

**Biochemical responses of maize (*Zea mays* L.)
to water stress**

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DECLARATION

I declare that the thesis entitled “Biochemical responses of maize (*Zea mays* L.) to water stress” has been prepared by me under the guidance of Professor Usha Chakraborty, Plant Biochemistry Laboratory, Department of Botany, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.



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This is to certify that Ms Rohini Lama is submitting her thesis entitled "Biochemical responses of maize (*Zea mays L.*) to water stress " on the basis of her work carried out under my supervision at the Department of Botany, University of North Bengal, for the award of Ph.D degree from the University of North Bengal

She bears a good moral character and I wish her all the best in life.

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ABSTRACT

Climatic factors, such as extreme temperatures (heat, cold, freezing), drought (deficient precipitation, drying winds), flooding, contamination of soils by high salt concentration (salinity) etc are major abiotic environmental stresses that limit plant growth and development, and thus agronomical yield. These different adverse but not necessarily lethal conditions are generally known as stress. Among them, water stress is one of the major constraints which affects productivity various crops all over the world.

Maize (*Zea mays* L.) is one the most important cereal crops of the world and is cultivated both in the spring and autumn season. However like every other crop the yield potential of maize is highly prone to various types of abiotic stresses, which has become a very important factor towards limiting the growth and production of maize. Water stress like drought and flooding during the summer season is one of the major production constraints for maize (*Zea mays* L.) in large areas of Southeast Asia. Considering this issue of maize production, identification and development of cultivars capable of withstanding the stress conditions could be an ideal and affordable approach suitable for resource to poor maize-growing farmers of such areas. The present study was undertaken in order to study the responses of seven cultivars of maize (*Zea mays* L.) to two different kinds of water stress-drought and flooding. The main objectives of the present study were to screen maize genotypes for their relative resistance tolerance to various degree of water stress -both drought and flooding. To study the biochemical parameters associated with water stress in terms of changes in the cellular constituents of leaf and roots of seven cultivars of maize including protein, carbohydrates, phenol, proline, chlorophyll. Determining the activities of antioxidative enzymes like catalase, peroxidase, ascorbate peroxidase, superoxide dismutase and glutathione reductase during water stress. To determine the relative water content and membrane damage of the leaves of seven test cultivars of maize during water stress and analysis of isozymes and proteins to develop markers of tolerance.

A review of literature pertaining to this investigation has been presented which deals mainly with the various types of abiotic stress giving special importance to water stress, its effects and current information about complex mechanisms underlying

drought and flood stress response and adaptation in higher plants especially maize. Standard methods were used for experimental purpose in order to achieve the above objectives.

Seven cultivars of maize (*Zea mays* L.) -KS 244, KS 244+, BN 101, 30V92, BN 1133, Swarna and Dhanya, were subjected to five sets of treatment in different stages in one set two month old plants of all the seven cultivars were grown in the glass house and was subjected to drought stress by completely withholding water in the pots of the test plants, and then the sampling of the plants were performed on the 3rd, 5th, and 7th day of drought stress along with the sampling of the well watered control plants. In the second set one week old seedlings of all the seven cultivars was subjected to drought stress by applying 10% (w/v) PEG-6000 (Polyethylene glycol), and sampling was done on the 3rd, 5th and 7th day of stress along with the sampling of the control plants which was done on 0-day. In the third set two week old plant of all the seven cultivars of maize that was grown *in vivo* was subjected to flooding stress by holding water. The test plants were then sampled on the 3rd, 5th and 7th day of stress. Control plants were sampled on the 0-day of stress and the fourth set one week old seedlings of two cultivars KS 244 and Dhanya (DMH 849) was transferred to a plot made in the field and was watered daily till the time of flowering, then the plants were subjected to drought stress by withholding water and drought subjected plants were sampled on the 20th day of drought stress along with the control which was sampled on 0-day of stress and the last fifth set was made by taking one month old plants of two cultivars KS 244 and Dhanya in pot culture and was given foliar spray of two chemicals ABA and proline of two different concentration each prior to drought stress and then various biochemical assays were performed on the treated and the untreated plant to see the ameliorating effects of the chemicals on the two cultivars.

These cultivars showed various degree of tolerance to drought and flooding stress. The seven cultivars of maize showed no morphological symptoms during the initial days of treatment but on prolonged water stress slight wilting was observed in all the seven cultivars. Initial screening of tolerant and susceptible cultivar was done by checking the relative water content and tolerance index from the leaves of the plant, which showed that KS 244, KS 244+, BN 101 were tolerant than the rest of the cultivars. Membrane damage during water stress was checked by electrolyte leakage

and lipid peroxidation in the leaves of the seven cultivars of maize, which showed that BN 1133, Dhanya and Swarna had the highest lipid peroxidation and membrane damage during water stress, especially when the plants were subjected to prolonged period of stress. Enzyme like POX, APOX, CAT, GR and SOD which are very important antioxidative enzymes, known to scavenge free oxide radicals during any kind of stress in plants showed various trends during various types of water stress. During *in vitro* drought stress POX activity in the leaves of KS 244, KS 244+ and BN 101 showed a slight decrease in activity on the 3rd day of drought stress, but again the activity increased on the 7th day of stress. In the other cultivars the activity declined drastically on the 7th day of stress. CAT and GR activity in the leaves of the test plants showed a similar trend in KS 244, KS 244+ and BN 101 where the activity showed a significant increase in the activity during drought stress. But in case of 30V92, BN 1133, Dhanya and Swarna a steep decline in the activity was observed during the period of stress. APOX and SOD activity in all the seven cultivars increased in the initial stages of stress but during prolonged stress a decrease in the activity was observed. During *in vivo* drought stress POX activity in the seven cultivars showed different trend in different cultivars. In KS 244+, KS 244 and BN 101 POX activity increased significantly with drought stress. But in BN 1133, 30V92, Swarna and Dhanya the activity decreased on the 7th day of stress. APOX activity increased in the initial period of stress and then decreased after the 7th day in all the cultivars. Similar type of trend was seen in case of SOD, where the activity decreased after the 5th day of stress in all the seven cultivars. GR and CAT activity in KS 244, KS 244+ and BN101 increased with the increase in drought stress but in the other four cultivars i.e BN 1133, 30V92, Swarna and Dhanya the GR and CAT activity showed very insignificant increase during the initial stages of drought with a decrease in the later stage of stress. In flooding stress POX activity showed an increasing trend in the activity in the initial days of flooding stress but gradually with prolonged stress the activity decreased in all the test cultivars. In KS 244, KS 244+ and BN 101 the activity decreased on the 7th day of stress and in BN1133, 30V92, Swarna and Dhanya the activity decreased from the 5th day of stress. CAT activity showed a gradual, significant increase in KS 244, KS 244+ and BN 101. But in BN 1133, 30V92, Swarna and Dhanya the catalase activity decreased during the period of stress. APOX activity and SOD activity in BN 101, KS 244, KS 244+ showed an increase in activity till the

5th day of flooding stress but a decrease in the activity was seen on the 7th day of stress a different trend was seen in case of BN 1133, Swarna, Dhanya and 30V92 where the APOX activity showed an initial increase and then decreased in activity after the 3rd day stress. The GR activity in KS 244, KS 244+ and BN 101 showed a significant increase in activity during the period of flooding stress. But like APOX and SOD, the GR activity showed a same type of trend in BN 1133, Swarna, Dhanya and 30V92 during flooding stress.

Apart from the antioxidative enzyme activities, the various antioxidants like ascorbate, carotenoids and α -tocopherol are also known to play important roles during plant stress. In the present study it was seen that there was an increase in the ascorbate and α -tocopherol content during water stress in the leaves of the test plants with maximum increase in the KS 244, KS 244+ and BN 101 cultivar. Over all a decrease in carotenoids were seen in all the seven cultivars of maize during water stress along with a decrease in chlorophyll content also. Maximum decrease in the chlorophyll content was seen in BN 1133, Dhanya and Swarna during water stress showing that these cultivars are very susceptible so to water stress than the rest of the cultivars.

Proline an important marker of drought stress was seen to increase during drought stress, and the increase was more in the leaves than in the roots of the test plants. Along with proline, sugar content in both the leaves and the roots of the test plant was also seen to increase during water stress. But a different trend was seen incase of starch content where unlike proline and sugar content, there was a decrease in the starch content in all the seven cultivars of maize during water stress. The decrease was seen both in the leaves and the roots of the test plants.

The total antioxidant activity in the leaves of the test plants was determined with respect to percent of DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) inhibition. During *in vitro* drought stress and *in vivo* drought stress it was seen that in cultivar KS 244, KS 244+ and BN 101, there was an increase in the DPPH inhibition during the period of stress. In case of 30V92 a decrease was seen on the 7th day of stress and in BN 1133, Dhanya and Swarna there was a slight increase on the 3rd day of stress but a significant decrease was seen on the 5th and the 7th day of water stress. In flooding stress KS 244, KS 244+, 30V92 and BN 101 increased during the period of stress but showed a slight decrease in the DPPH inhibition on the 7th day of stress. The other

cultivars showed a significant decrease in the DPPH inhibition during the period of stress.

H₂O₂ level in leaves of the tolerant cultivars did not show much increase during water stress, along with a slight decrease on prolonged period of stress, but in case of the susceptible cultivar the H₂O₂ levels were seen to show highly significant increase on prolonged period of water stress.

During water stress a decrease in the phenolic content from the leaves was observed in all the seven cultivars of maize which matched with the results of HPLC analysis of total phenol and phenolic acids of two cultivars of maize-KS 244 and Dhanya during *in vivo* drought and flooding stress which showed that some of the peaks corresponding various total phenols and phenolic acid disappeared in the test plants subjected to water stress. There was also a significant decrease in the height of the peaks in the plants subjected to stress in comparison to the control. Protein profile analyzed by SDS-PAGE, showed the expression of some new protein bands in the leaves of most of the cultivars drought and flooding stress *in vivo*. Peroxidase isozyme analysis also revealed the presence of new bands during water stress in these cultivars. Amelioration of drought stress *in vivo* in two cultivars of maize- KS 244 and Dhanya by foliar application of two chemicals ABA (50µM and 100µM) and proline (30mmol and 60mmol) showed that foliar application of ABA and proline was quite effective in ameliorating the adverse effects of water stress in maize by enhancing defense mechanisms and the best results were seen in case of 30mmol proline application. Thus results of all experiments revealed that out of the seven test cultivars of maize-KS 244, KS 244 and BN 101 are the more tolerant cultivars to both types of water stresses and BN 1133, Dhanya and Swarna are the susceptible cultivars to both type of water stresses and it may also be stated that the oxidative stress induced by water stress in maize cultivars is countered by the over production of stress responsive metabolites, antioxidants and antioxidative enzymes, specially in the cultivars which could withstand water stress more efficiently.

PREFACE

Maize is one of the common cultivated crops of both plains and hills of North Bengal. Among various abiotic stresses, excessive soil moisture caused by flooding, water logging or a high water table due to excessive rainfall and /or inadequate drainage is one of the major constraints for maize production and productivity in Asian regions. Considering the above, the present work is envisaged to determine how maize plants respond to water stress-excess and deficit, their mechanisms of action.

It has been a great experience to carry on my research work in the Department of Botany, University of North Bengal. As I cross another milestone in my life I wish to take this opportunity to thank all those who were of valuable help and guidance for the successful completion of my thesis, I would like to pen down some words with all gratitude and praise to the Lord.

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Stress is a concept imported from physics. It was introduced in the theory of elasticity as the amount of force for a given unit area (Kranner and Minibayeva 2010). In biological context, stress is usually defined as an external factor that exerts a disadvantageous influence on the plant (Taiz and Zeiger 1991). Alternatively, stress could be defined as a significant deviation of the optimal condition of life (Larcher 2003). Plants in nature may be exposed, during their ontogeny, to a wide cultivar of favorable or disadvantageous biotic and abiotic factors. The biotic and abiotic relationships that have developed determine the existence, structure and survival of plants in the case one or several components of the system change. To maintain its functional and structural integrity, a plant organism has to be resistant towards unfavorable factors. Each organism has a unique range of genetically determined and phylogenetically adapted physiological resistance within which a factor affecting it is tolerable. If a stress factor surpasses this range the plant has to trigger additional energy and physiological–biochemical mechanisms to survive under unfavorable conditions. Such case is referred to as stress and stress factors (Mandre 2002). Plants adapt to their ever changing environment in many ways, leading to a wealth of growth forms of varying complexity. Certain habitats demand exceptional adaptations, especially when one or more essential resources are scarce or absent (Huang *et al.* 2001). Abiotic stress is defined by Skirycz and Inze (2010); Cramer (2010) as environmental conditions that reduce growth and yield below optimum levels. Plant responses to abiotic stresses are dynamic and complex (Skirycz and Inze 2010; Cramer 2010). Abiotic stresses such as extremes of temperature and pH, high salinity and drought, comprise some of the major factors causing extensive losses to crop production worldwide. Understanding how plants respond and adapt at cellular and molecular levels to continuous environmental changes is a pre-requisite for the generation of resistant or tolerant plants to abiotic stresses (Pinho dos Reis *et al.* 2012). Stressful environments such as salinity, drought, and high temperature (heat) cause alterations in a wide range of physiological, biochemical, and molecular processes in plants. These stressful environments, including drought, salinity, and unfavorable temperatures, considerably hamper the process of photosynthesis in most plants by altering the ultra structure of the organelles and concentration of various

pigments and metabolites including enzymes involved in this process as well as stomatal regulation (Ashraf and Harris 2013).

Environmental stresses, such as drought, salinity, cold and heat cause adverse effects on the growth of plants (Bray *et al.* 2000; Griffiths and Parry 2002) and affects almost all plant functions (Griffiths and Parry 2002; Hernandez *et al.* 2001) metabolism and yield (Reddy *et al.* 2004). At the molecular level, this response also includes the expression of stress-inducible genes involved in direct plant protection against stress (Chinnusamy *et al.* 2004; Shinozaki *et al.* 2007; Krasensk and Jonak 2012). In all natural habitats, plants are surrounded by an enormous number of potential enemies (biotic) and various kinds of abiotic environmental stress. Nearly all ecosystems contain a wide cultivar of bacteria, viruses, fungi, nematodes, mites, insects, mammals and other herbivorous animals, greatly responsible for heavy reduction in crop productivity (Mazid *et al.* 2011). All environmental stresses, irrespective of their targets and nature of perception bring perturbation in the cellular energy homeostasis. The stress-adaptive mechanisms developed by these photosynthetic organisms are primarily based on re-establishment of cellular energy balance (De Block *et al.* 2005; Dobrota 2006). Thus environmental stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50%. Among the abiotic factors that have shaped and continue shaping plant evolution, water availability is the most important. Water stress in its broadest sense encompasses both drought and salt stress. Drought and salinity are becoming particularly widespread in many regions, and may cause serious salinization of more than 50% of all arable lands by the year 2050 (Bray *et al.* 2000; Anjum *et al.* 2011a). Overall, stress tolerance is a complex phenomenon because plants may go through multiple stresses at the same time during their development (Mahajan and Tuteja, 2005). Adaptation represents the ability of living organisms to fit into a changing environment, at the same time improving its chances of survival and reproducing itself (Fujita *et al.* 2006). Water stress due to drought and salinity is probably the most significant abiotic factor limiting plant and also crop growth and development (Hartman *et al.* 2005). Salinity and drought stresses are physiologically related, because both induce osmotic stress and most of the metabolic responses of the affected plants are similar to some extent (Djibril *et al.* 2005). The natural environment for plants is composed of a complex set of abiotic stresses and biotic

stresses. Plant responses to these stresses are equally complex (Cramer *et al.* 2011). In addition, the level and duration of stress can have a significant effect on the complexity of the response (Tattersall *et al.* 2007; Pinheiro and Chaves 2011). Among the abiotic stresses, drought is the most severe limitation to maize production (Sallah *et al.* 2002). To a careful estimate, only drought reasons for 50% or more reduction in average yields worldwide (Wang *et al.* 2003). Drought causes numerous physiological and biochemical changes in plants like reduced leaf size, stem extension, root proliferation, reduced water use efficiency (Farooq *et al.* 2009). There is ample evidence that ROS are also crucial second messengers involved in the response to diverse abiotic and biotic forms of stress. An oxidative burst takes place in response to abiotic stress factors (Wahid *et al.* 2007; Kwon *et al.* 2007; Miller *et al.* 2010). α -tocopherol, found in green parts of plants scavenges lipid peroxy radicals through the concerted action of other antioxidants (Kiffin *et al.* 2006). Proline which known to occur widely in higher plants, normally accumulates in large quantities in response to environmental stresses (Kavikishore *et al.* 2005; Ashraf and Foolad 2007). The antioxidant defense machinery protects plants against oxidative stress damages. Plants possess very efficient enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaiacol peroxidase, GOPX and glutathione-S- transferase, GST) and non-enzymatic (ascorbic acid, ASA; glutathione, GSH; phenolic compounds, alkaloids, non-protein amino acids and α -tocopherols) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS. ROS also influence the expression of a number of genes and therefore control the many processes like growth, cell cycle, programmed cell death (PCD), abiotic stress responses, pathogen defense, systemic signaling and development (Gill and Tuteja 2010). A remarkable feature of plant adaptation to abiotic stresses is the activation of multiple responses involving complex gene interactions and crosstalk with many molecular pathways (Basu 2012; Umezawa *et al.* 2006).

Maize is one of the most important cereals cultivated in the world, since it presents high potential for grain production, nutritional value, and chemical composition (Zaidi *et al.* 2007a). It is used as food, feed, fodder, fuel and in the manufacture of industrial

product (Liu *et al.* 2001). Among cereals, maize (*Zea mays* L.) not only has a sufficient amount of carotenoids, tocopherols, and oil but also has a reasonable amount of starch and protein content compared with other major food crops such as rice and wheat. Although maize is principally cultivated for carbohydrate production, in the past several years, it has gained great significance as a source of vegetable oil for the food industry (Balasundram *et al.* 2006). Maize oil is considered the best as a vegetable oil due to its large amount of unsaturated fatty acids, most predominantly being oleic and linoleic acids ranging from 65 to 85% (Goffman and Böhme 2001). Despite a large amount of unsaturated fatty acids, the oil is a rich source of phenolics, flavonoids, and different types of tocopherols (Balasundram *et al.* 2006). Maize is cultivated in both spring and autumn seasons and it is best suited in existing cropping scheme. However, yield potential of maize is highly prone to abiotic stresses (drought, salinity, extreme temperatures, flooding, pollutants & poor or excessive irradiation) (Lawlor 2002). Among various constraints responsible for low grain yield, inadequate supply of water at its critical development stage and high sensitivity of different maize cultivars to water stress are of immense importance (Shakhathreh *et al.* 2001) thus leading to the most severe limitation to maize production (Sallah *et al.* 2002). Changes in the level of reactive oxygen species and antioxidants were investigated in maize (*Zea mays* L.) subjected to abiotic stress (Kellôs *et al.* 2008). Many abiotic stresses directly or indirectly affect the synthesis, concentration, metabolism, transport and storage of sugars. Soluble sugars act as potential signals interacting with light, nitrogen and abiotic stress (Obertello *et al.* 2010; Osuna *et al.* 2007). The inability of maize plants to endure low oxygen availability in the rhizosphere, caused by root flooding, result in substantial losses in productivity for example in South Asia, 15% of maize acreage is affected by flooding, whereas in India, water excess is already the second major stress, reaching around 8.5 million hectares (Zaidi *et al.* 2007a).

Considering the importance of abiotic stresses in maize production, the present study has been undertaken to understand, in detail, the biochemical mechanisms involved in tolerance/susceptibility of maize cultivars to both drought and flooding. The major objectives of the work are:

- ❖ To screen maize genotypes for their relative tolerance to various degree of water stress.
- ❖ To study the biochemical parameters associated with water stress in terms of changes in the cellular constituents of leaf and roots including protein, carbohydrates, phenol, proline, and chlorophyll.
- ❖ To determine total antioxidant activities following stress
- ❖ Determination of activities of antioxidative enzymes like catalase, peroxidase, ascorbate peroxidase, superoxide dismutase and glutathione reductase and accumulation of small antioxidants like ascorbate, carotenoids and tocopherol.
- ❖ Determination of membrane damage following water stress.
- ❖ Analysis of isozymes and proteins to develop markers of tolerance.
- ❖ To determine whether water stress can be ameliorated by compounds such as ABA and proline and the mechanisms thereof.

The present literature survey deals with the current information about complex mechanisms underlying drought and flood stress response and adaptation in higher plants especially maize (*Zea mays* L.). These include biochemical changes during stress, gene expression and transcriptional regulation of stress related genes and the mechanism of cross tolerance across plant genera.

2.1. Effects of water deficit on plant system

Plants in nature are continuously exposed to several biotic and abiotic stresses. Among these stresses, drought stress is one of the most adverse factors of plant growth and productivity and considered a severe threat for sustainable crop production in the conditions on changing climate. Drought triggers a wide cultivar of plant responses, ranging from cellular metabolism to changes in growth rates and crop yields. Understanding the biochemical and molecular responses to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions (Anjum *et al.* 2011). Water deficit (commonly known as drought) can be defined as the absence of adequate moisture necessary for normal plant grow and to complete the life cycle (Zhu 2002). It is one of the most common environmental limitations affecting growth and productivity of plants, and causes many metabolic and oxidative changes in plants (Reddy *et al.* 2004; Kalefetog˘lu and Ekmekc 2005; Xiao *et al.* 2009; Efeoglu *et al.* 2009) and recent global climate change has made this situation more serious (Pan *et al.* 2002). Water is already a scarce commodity in many parts of the world, and predicted climate changes will aggravate the situation in future. In addition to decreases in the amount and frequency of precipitation, global increases in temperature are predicted to enhance water losses further through evapo-transpiration (IPPC 2007). The lack of adequate moisture leading to water stress is common occurrence in rain fed areas, brought about by infrequent rains and poor irrigation (Wang *et al.* 2005). The increased irrigation required to meet the needs of crop growth is expected to lead to groundwater depletion (Konikow and Kendy 2005; Oki and Shinjiro 2006). During the acclimation to soil drought, plants initiate defence mechanisms against water deficit (Medrano *et al.* 2002; Chaves and Oliveira 2004). Responses to drought are species specific and often genotype specific (Campos *et al.* 2004). The reaction of plants to water stress differs significantly at various

organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development (Demiral and Turkan 2005). Drought means water loss and dehydration at normal or even elevated temperatures. Shrinking of cells leads to loss of turgor, osmotic stress and a potential change of membrane potentials. Upon severe water loss from the cells, membrane disintegration and abolition of metabolic processes occur (Mahajan and Tuteja 2005).

Numerous studies have demonstrated that adverse conditions (water deficiency) can induce membrane damage, increase membrane permeability and the accumulation of free radicals in plants. As a reaction to the adverse conditions, anti-oxidative enzymes and small molecule substances can be produced to remove those active oxygen radicals (Wang *et al.* 2002; Sun *et al.* 2003). Water stress causes stomatal closure, which reduces the CO_2/O_2 ratio in leaves and inhibits photosynthesis (Jason *et al.* 2004; Moussa 2006). The plant responses are dependent on the tissue or organ affected by the stress. For example, transcriptional responses to stress are tissue or cell specific in roots and are quite different depending on the stress involved (Dinneny *et al.* 2008). In addition, water deficit alters the cell wall non-enzymatically; for example, by the interaction of pectate and calcium (Boyer 2009). Drought causes numerous physiological and biochemical changes in plants like reduced leaf size, stem extension, root proliferation, reduced water use efficiency (Farooq *et al.* 2009), alteration in metabolic activities (Lawlor & Cornic 2002). The availability of water (in addition to atmospheric CO_2) is considered to have been a major driving force for the evolution and the ecological success of C4 plants (Edwards *et al.* 2010; Westhoff and Gowik 2010). Water deficit inhibits plant growth by reducing water uptake into the expanding cells, and alters enzymatically the rheological properties of the cell wall; for example, by the activity of ROS (reactive oxygen species) on cell wall enzymes (Skirycz and Inze 2010). The initial growth inhibition by water deficit occurs prior to any inhibition of photosynthesis or respiration (Hummel *et al.* 2010). Drought stress can cause primarily the formation of reactive oxygen species (ROS) and the alteration of water potential within the plant (Rodriguez *et al.* 2010). However in certain tolerant/adaptable crop plants morphological and metabolic changes occur in response to drought, which contribute towards adaptation to such unavoidable environmental constraints (Ben Amor *et al.* 2007). In the cellular level, membranes and proteins can be damaged by a reduction in hydration and increase of reactive

oxygen species (ROS) (Artlip & Wisniewski 2002; Castrillo *et al.* 2001). There are different approaches to mitigate the drought hazards, which include the development of stress tolerant plants by selection of stress resistant varieties (Ahloowalia *et al.* 2004). Water deficit affects the germination of seed and the growth of seedlings negatively (Van den Berg and Zeng 2006). The nature of drought responses of plants is influenced by the duration and severity of water loss (Pinheiro and Chaves 2011). Under severe drought stress the root growth was severely reduced in maize plants (Ali *et al.* 2011). Studies done by (Ali *et al.* 2010) revealed that highest root fresh and dry weights were observed in maize plants exposed to mild drought stress whereas, severe drought stress markedly reduced root fresh and dry weights. An increase in root fresh and dry weight of maize plants under the conditions of mild water stress (Hamayun *et al.* 2010) is an important adaptive feature due to assimilation of photosynthates used in the stem for increasing the root growth and increase in root respiration efficiency. While under severe drought stress the root growth was severely reduced due to reduced root penetration into dry soil and physical damage to young root tips a potential source of cytokinins. (Nejad *et al.* 2010; Ahmed *et al.* 2008 ; Abdalla and El-Khoshiban 2007) reported that water stress increased the growth of roots but decreased those of shoots, number and area of leaves, fresh and dry weights of shoots and roots, the percentage of dry matter and the spike weight of wheat plants. However, water stress that exists at the reproductive stage severely affects grain yield of mung bean more than its occurrence at other stages (Thomas *et al.* 2004). Leport *et al.* (2006) found that pod production of chickpea was more affected by early podding water stress than by late podding water stress. Tolerance to abiotic stresses is very complex at the cellular levels of the whole plant (Foolad *et al.* 2003a, b; Ashraf and Harris 2004).

2.1.1. Morphological effects and relative water content

Tawfik (2008) performed experiment on mung bean showed that drought stress caused an observed adverse action on plant growth and productivity. Fresh and dry weights were significantly reduced under water stress. Growth criteria were higher for plants subjected to extension of irrigation interval for two days compared to five and ten days extension. Plants irrigated every ten days produced only 36.4% and 38.1% fresh and dry weights, respectively, compared to the control at 90 days old. Plants treated with potassium and irrigated every two days recorded, 119.5% and 121.7%

fresh and dry weights, respectively, compared to the control at 90 days old, while fresh and dry weights of plants irrigated every ten days were reduced to 51.4% and 56.1%, respectively, compared to the control. These observations indicated that addition of potassiomag biofertilizer mitigated the harmful effect of water stress.

Experiments performed by Anjum *et al.* (2011a) on maize plants which were exposed to drought stress at teaseling stage, showed a substantial reduction in yield and yield components such as kernel rows/cob, kernel number/row, 100 kernels weight, kernels/cob, grain yield/plant, biological yield/plant and harvest index (drought led to substantial impairment of growth related traits of maize in terms of plant height, leaf area, number of leaves/plant, cob length, shoot fresh and dry weight/plant).

Water stress causes water loss within the plant and therefore a reduction in its relative water content. In this sense, one of the most reliable and widely used indicators for defining both the sensitivity and the tolerance to water stress in plants is leaf relative water content (Rodriguez *et al.* 2010) he reported that RWC was one of the best indicators in tomato plant for separating tolerant and sensitive cultivars.

Yang and Miao (2010) studied that when two poplar species were submitted to progressive drought stress, the decrease of RWC in the water-stressed cuttings was 23.3% in *Populus cathayana*, whereas it was 16% in *Populus kangdingensis*. RWC was affected by the interaction of severity, duration of the drought event and species.

The effects of sodium nitroprusside (SNP; nitric oxide donor) treatment on induced drought stress were investigated by Shehab *et al.* (2010). Drought stress induced by polyethylene glycol (PEG) with different concentrations 5%, 10%, 15% and 20% and previous four concentrations of both poly ethylene glycol and 100 μ M of SNP on rice (*Oryza sativa*) culture were used. The results showed that, while drought conditions increased after four weeks of administration, the stress signals increased markedly via H_2O_2 and MDA as a response to elevated oxidative damage promoted by lipid peroxidation under elevated free radical formation.

In experiments performed by Ali *et al.* (2010) in *Kochia* (*Kochia scoparia*) under saline condition the RWC of the leaves declined by 20.5%, while the EL increased by 50% at stress treatment compared with control, but there were no significant differences in RWC and EL between control and recovered plants. Birjand ecotype had more RWC (64%) and least EL (53%) compared with Borujerd with RWC (59%)

and EL (66%) on stress situation, respectively. Experiments performed on bread wheat by Keyvan (2010) showed cultivar and drought stress made an effect on the changes of flag leaf RWC and it was very significant statistically. In various wheat cultivars, the mean comparison mean of flag leaf RWC showed Chamran cultivar with 73.58 percent has highest flag leaf RWC and Shahryar cultivar with 67.33 percent had lowest. Also, in different treatments of stress, the mean comparison of flag leaf RWC showed drought stress treatment with 47.89 percent had highest decrease of RWC than control treatment with 93.56 percent.

Arjenaki *et al.* 2012, performed experiments in order to evaluate drought stress on relative water content (RWC), mineral elements and chlorophyll content of wheat varieties, an experiment based on randomized complete block design with three replications was conducted in 2008 in Karaj region, Iran. Treatments were 6 wheat varieties Sardari, Kavir, Varinac (asresistant var.), Marvdasht, Tajan and Ghods, (as susceptible var.). Drought stress was applied by withholding water at anthesis stage. Drought on genotypes significantly ($p < 0.01$) affected RWC. Kavir and Sardari genotypes had the highest values of 79.96, 74.43%, respectively. Ghods genotype had the lowest RWC of 59.3%.

Five varieties of wheat (*Triticum aestivum* L.) KW, UP 2752, PBW 343, SO and LV - were subjected to water stress and sampling was done on the 3rd, 6th and 9th day of stress by Chakraborty and Pradhan (2012). The plants of all the five varieties under test showed less or no symptoms of wilting till the 6 to 7th day of stress, however severe wilting occurred in the plants after 9th days of stress more pronounced in varieties LV and SO. RWC in the leaf of all the varieties declined remarkably as the severity of stress in terms of days was increased. Data showed that the decrease in RWC was significantly lower in case of PBW 343, UP 2752 and KW on the 9th day of stress when compared to their respective controls (40.75, 42.34 and 36.65%, respectively) however the decline in RWC was much more in LV and SO with respect to their controls (52.93 and 52.67%,) respectively.

In order to assess drought tolerance mechanism in cotton, short-term drought-induced biochemical responses were monitored in two cotton (*Gossypium hirsutum* L.) genotypes contrasting their tolerance to water deficit by Parida *et al.* 2007. The seeds of two genotypes, namely GM 090304 (moderately drought tolerant) and Ca/H 631

(drought sensitive) were taken by them. RWC of leaves were 75% and 68.4% in GM 090304 and Ca/H 631 genotypes, respectively, under control conditions, while the drought stress caused a decrease in RWC to 72% and 63.7% in GM 090304 and Ca/H 631 genotypes, respectively. However, on re-watering, the plants recovered fully in terms of RWC.

2.1.2. Membrane damage and electrolyte leakage

Drought stress is also known to result in an extensive lipid peroxidation. MDA is the product of lipid peroxidation and usually used as the criterion of stress-induced oxidative damage to membrane (Zhao and Tan 2005). The ROS is potentially harmful to cell membranes, resulting in oxidative degradation of membrane lipids (lipid peroxidation). Malondialdehyde (MDA) is one of the end breakdown products of lipid peroxidation and can be used as an indicator of in vivo lipid peroxidation (Xue and Liu 2008). The MDA content in root and leaves of *L. esculentum* ‘Solan Sindhur’ increased under drought stress. The observed increase of MDA under drought stress condition results from production of reactive oxygen species (ROS) which lead to lipid peroxidation (Kumar *et al.* 2011). Earlier studies report that MDA content increase under PEG stress (Gengsheng *et al.* 2001).

Simova-Stoilova *et al.* (2009) performed experiments to study the antioxidative protection in leaves of four winter wheat (*Triticum aestivum* L.) varieties with different field drought resistance was studied under severe recoverable soil drought at seedling stage by withholding irrigation for 7 days (57-59% leaf water deficit) followed by rewatering. A 3-fold raise in electrolyte leakage corresponded to drought severity. Hydrogen peroxide content and CAT activity were maintained low under stress. As a result of the treatment, the electrolyte leakage from cell membranes, reflecting membrane intactness, increased about 3-fold. This damage was recoverable after 3 days of optimal water supply for all varieties under study except the most drought-sensitive one (Miziya). Hydrogen peroxide content was low under drought compared to 7-day old control plants. Values of the recovered plants were comparable to the respective controls. Malondialdehyde level was not changed significantly in the leaves of drought treated plants but raised during recovery.

The rise in MDA content under stress conditions suggests that water stress could induce membrane lipid peroxidation by means of ROS (Sairam *et al.* 2000). Yang and

Miao (2010) noted the increments of the MDA and H_2O_2 concentrations in the water-stressed cuttings were 88.9 and 99.7% in *P. cathayana*, respectively, whereas they were only 44 and 63.6% in *P. kangdingensis*.

Terzi and Kadioglu (2006) studied the relationship of the antioxidant enzyme system to drought stress tolerance during leaf rolling in the leaf, petiole and root of *Ctenanthe setosa* (Rosc.) Eichle. Lipid peroxidation, measured as MDA content, increased up to day 40 of drought in the leaf and petiole but decreased later. For example, MDA content was 30.8 nmol g f.w. in control leaves, 46.5 nmol g f.w. in 40-day drought plants and 25.1 nmol g f.w. in 64-day-drought plants. In the root, however, lipid peroxidation increased up to day 48 of drought.

The accumulation of H_2O_2 and lipid peroxidation increased with increase in the days of stress. The accumulation was higher in case of LV, SO following water stress and lower in case of KW, UP 2752 and PBW 343. Lipid peroxidation is measured in terms of MDA content which during water stress was found to be thrice in SO and LV than in KW, UP 2752 and PBW 343. It also showed a general increase with the increase in the severity of water stress with respect to their respective controls in five varieties of wheat (*Triticum aestivum* L.) - KW, UP 2752, PBW 343, SO and LV - which were subjected to water stress and whose sampling was done on the 3rd, 6th and 9th day of stress by Chakraborty and Pradhan (2012). Thus Lipid peroxidation, which refers to the oxidative degradation of lipids, can be a widely used marker of oxidative damage (Uzilday *et al.* 2012).

Sharma and Dubey (2005) performed experiments on rice seedlings which were grown for 10 and 20 days and then was subjected to *in vitro* drought stress of -0.5 and -2.0 MPa for 24 h. Due to imposition of mild as well as high drought stress, a significant increase in superoxide anion production was observed in roots and shoots of both the rice cvs. Malviya-36 and Pant-12. Between 114 and 167% increase in the concentration of O_2^- was observed in highly drought stressed seedlings grown for 20 days compared to unstressed seedlings. However, the concentration of H_2O_2 declined in the seedlings under drought stress. A greater decline in H_2O_2 was observed in mild drought stressed seedlings. A decline in H_2O_2 concentration of between 12 and 40% was observed in 20 days grown mild drought stressed rice seedlings compared to control seedlings.

Experiment performed by Jamwal *et al.* (2012) to study the impact of water-deficit stress on the seed germination and growth of *Lycopersicon esculentum* ‘Solan Sindhur’ showed that leaf and root MDA content increased with increase in PEG concentration. But increase in MDA content in leaf was much more as compared to root. MDA content present in leaf was 27.46%, 40.45%, 46.61% and 52.83% higher than the control at 1% PEG, 5%PEG, 10% PEG and 15% PEG treatments, respectively. Likewise the root MDA content was 34.41%, 46.82%, 58.52% and 75.41% higher than control at 1%, 5%, 10% and 15% PEG treatments, respectively.

Hosseini Boldaji *et al.* (2012) performed experiments to understand alfalfa (*Medicago sativa* L.) reactions to osmotic stress, solutions with -0.5 , -1 and -1.5 MPa osmotic potentials using PEG (Poly ethyleneglycol) and distilled water as control. In a germination test, eleven alfalfa cultivar seeds were allowed to germinate in these solutions. *M. sativa* cv. Yazdi and *M. sativa* cv. Gharayonje, selected as tolerant and sensitive cultivars, respectively, and were used for further studies. In all PEG solutions, root and shoot dry weights decreased in both cultivars. Under different levels of osmotic stress, root to shoot ratio increased significantly in Yazdi, whereas this parameter showed no significant differences in Gharayonje. Yazdi cultivar also showed higher activities of SOD, APOX, CAT, POX and higher reducing sugar contents of leaves in comparison with Gharayonje. These higher antioxidant activities help the tolerant cultivar to decrease oxidative damages of osmotic stress to membrane lipids as compared with its sensitive counterpart. As a result, electrolyte leakage and the amounts of MDA (Malondialdehyde), were higher in Gharayonje.

Seven species of genus *Avena* viz., *Avena sativa*, *Avena strigosa*, *Avena brevis*, *Avena vaviloviana*, *Avena abyssinica*, *Avena marocana* and *Avena sterilis* were used to study the impact of drought stress on lipid peroxidation and other antioxidant enzymes by Pandey *et al.* (2012). Level of lipid peroxidation in terms of Malondialdehyde (MDA) content was increased in the leaves when plants were subjected to moisture stress. The rate of increase in lipid peroxidation occurred irrespective of stage however; maximum increase was recorded in *A. strigosa* at both the stages. *Avena* species which showed high level of MDA content, indicates more lipid peroxidation and more membrane permeability and are comparatively more susceptible for water stress than those which produce less MDA at higher magnitude of water stress and such species have better capability for moisture and stress tolerance. At vegetative

stage maximum and minimum value was recorded in *A. strigosa* (114.25 ± 1.395) nano moles g-1dw. and *A. vaviloviana* (167.86 ± 0.215) nano moles g-1dw respectively. However over extreme stress environment the value ranges from 161.64 ± 0.00 nano moles g-1dw. in *A. brevis* to *A. sterilis* (199.64 ± 0.644) nano moles g-1dw. The percent of increase in the MDA content varies significantly among the species tested. The maximum percent of increase over control was recorded in *A. strigosa* (54.86%) followed by *A. sterilis* (25.51%) and *A. sativa* (25.27%) and re-watering the MDA content again decreased to the tune of controlled environment. At flowering stage under controlled environment maximum MDA accumulation was recorded in *A. abyssinica* (202.37 ± 0.48) nano moles g-1dw followed by *A. vaviloviana* (200.28 ± 0.644) nano moles g-1dw and *A. sterilis* (199.3 ± 1.932) nano moles g-1dw. Under extreme stress the MDA content increased and the maximum value recorded was 334.39 ± 0.483 nano moles g-1dw. in *A. strigosa*, followed by *A. brevis* ($282.23 \pm$) nano moles g-1d wt. and *A. sterilis* (265.16 ± 1.449) nano moles g-1dw. The increase in the MDA content varies from species to species. Maximum percent of increase in the MDA content over control was recorded in *A. strigosa* (68.31%) followed by *A. brevis* (56.52%) and *A. sativa* (38.87%). On re-watering the value of MDA again decreased to tune of the plants grown under controlled environment.

High concentrations of malondialdehyde increases lipid peroxidation and oxidation of cell membrane fatty acids reactions, which finally decreases cell membrane stability index (Hong *et al.* 2006; Dacosta and Hoang 2007).

Two shrubs, *Pyracantha fortuneana* and *Rosa cymosa*, and four trees, *Broussonetia papyrifera*, *Cinnamomum bodinieri*, *Platycarya longipes* and *Pteroceltis tatarinowii*, were randomly assigned to four drought treatments by Liu *et al.* (2011) in order to see the effect of drought on pigments, osmotic adjustment and antioxidant enzymes. After 20 days of treatments, MDA content and ion leakage in all six species gradually increased as drought stress intensified. Prolonged drought treatments usually increased MDA content and ion leakage in all six species, except MDA content in *P. fortuneana*. After 40 days of severe drought, *P. fortuneana*, *R. cymosa* increased as drought intensified. However, CAT activity in *C. bodinieri* and *B. papyrifera* increased only under moderate stress and in *P. tatarinowii* under mild stress. Prolonged treatments significantly increased CAT activity in all six species except *P. tatarinowii* under mild and/or moderate stress, but decreased that in *C. bodinieri* and

P. tatarinowii under severe stress. After 20 days of treatments, POX activity showed highest values in *P. fortuneana*, *R. cymosa* and *B. papyrifera* under severe stress and in the other three species under moderate stress. Prolonged treatments increased POX activity in *P. fortuneana*, *C. bodinieri*, *B. papyrifera* and *P. tatarinowii* under mild and/or moderate stress, increased that in *P. fortuneana* under severe stress, but decreased that in *R. cymosa*, *C. bodinieri*, *B. papyrifera* and *P. tatarinowii* under severe stress.

2.1.3. Antioxidative enzymes and antioxidants

Exposure of plants to most adverse conditions causes oxidative stress, which affects plant growth due to the production of Reactive Oxygen Species (ROS) such as superoxide radicals ($\text{O}_2^{\cdot-}$), singlet oxygen ($^1\text{O}_2$), hydroxyl radicals ($\cdot\text{OH}$) and hydrogen peroxide (H_2O_2) (Mittler 2002). Plants have developed different scavenging mechanisms to control the level of ROS by an interacting network of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Xue and Liu, 2008). A close relationship between antioxidant activity and stress tolerance has been identified in several crops (Zhang *et al.* 2003; Zhang & Ervin 2004; Misra and Gupta 2006). The most important antioxidant enzymes are superoxide dismutase (EC 1.15.1.1; SOD), ascorbate peroxidase (EC 1.11.1.11; APX), glutathione reductase (EC 1.6.4.2; GR) and catalase (EC 1.11.1.6; CAT) (Mittler 2002). Water stress breaks down the balance between the production of reactive oxygen species (ROS) and the antioxidant defense system causing the accumulation of ROS which induces oxidative stress to protein, membrane lipids and disruption of DNA strands (Gong *et al.* 2005, El-Tayeb 2006). Mechanisms of active oxygen species detoxification exist in all the plants and include activation of enzymes (Johnson *et al.* 2003) as well as non-enzymatic (flavones, anthocyanins, carotenoids and ascorbic acid) antioxidants (Nayyar and Gupta 2006). SOD is the first enzyme involved in the antioxidative process (Rubio *et al.* 2002) it is a class of enzymes that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. As such, it is an important antioxidant defense in nearly all cells exposed to oxygen (Corpas *et al.* 2006) and plays a key role in the reduction of oxidative damages. (Zhang *et al.* 2005; Gr  o *et al.* 2005); SOD converts superoxide to H_2O_2 and O_2 . H_2O_2 is then scavenged by CAT and a cultivar of peroxidases through the oxidation of co-substrates such as phenolics or other antioxidants (Basu *et al.* 2010) Catalase is a common enzyme found in nearly all

living organisms that are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Vertuani *et al.* 2004). Catalase has one of the highest turnover number of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Chelikani *et al.* 2004). Catalase activity responds to drought in many ways: it can increase, decrease or remain unchanged (Zgallaï *et al.* 2006). Various associations between water deficit stress and endogenous levels of water soluble antioxidants have been described (Borrmann *et al.* 2009; Manavalan *et al.* 2009).

Terzi and Kadioglu (2006) studied the relationship of the antioxidant enzyme system to drought stress tolerance during leaf rolling in the leaf, petiole and root of *Ctenanthe setosa* (Rosc.). Superoxide dismutase (SOD) activity did not significantly change in leaves. In the petiole and root, however, it decreased in the early drought period but increased later. Glutathione reductase (GR) activity did not significantly change in the leaf and petiole versus the control, but increased in root. Peroxidase (POX) activity increased in the leaf and petiole but decreased in the root. A peroxidase isoenzyme activity band present in the control leaves did not appear in leaves exposed to 32 days of drought, but in the later periods that activity increased. Tolerance of drought stress apparently is closely associated with the antioxidant enzyme system as well as leaf rolling in *C. setosa*.

Abedi *et al.* (2010) also undertook studies to identify the responses of antioxidant enzyme activities and their isozyme patterns in seedlings of 10 oilseed rape (*Brassica napus* L.) cultivars under drought stress conditions. Plants were grown under three irrigation regimes (FC; field capacity, 60% FC and 30% FC) in a greenhouse. Drought stress preferentially enhanced the activities of superoxide dismutase and guaiacol peroxidase whereas it decreased catalase activity. Licord with the highest level of enzyme activity under both optimum and limited irrigation regimes is reported as the most tolerant cultivar, whereas Hyola 308 and Okapy, having the lowest enzymes activities, are mentioned as cultivars sensitive to drought stress. The native polyacrylamide gel electrophoresis analysis detected eight SOD isozymes. Oilseed rape leaves contained three isoforms of Mn-SOD and five isoforms of Cu/Zn-SOD. The expression of Mn-SOD was preferentially enhanced by drought stress. Five POD isoforms were detected in oilseed rape leaves. The intensities of POD-4 and -5 were enhanced under drought stress. According to the results, the appearance of new

isozyme bands under drought stress conditions may be used as a biochemical marker to differentiate drought tolerant cultivars under drought stress. The results revealed an increase in SOD and POX activities and a decrease in CAT activity in leaves of oilseed rape under drought treatment.

In studies done by Qiang *et al.* (2011) CAT activities in both cultivars of Jerusalem artichoke increased under drought treatments, with cv. Xiuyan showing a significant increase in CAT activity as compared to cv. Yulin, suggesting that CAT may be responsible for the better protection against oxidative damage in cv. Xiuyan. Drought stress also led to a relatively higher increase of SOD activity in cv. Xiuyan than cv. Yulin, which meant that SOD activity also associates with the better protection against oxidative damage in Jerusalem artichoke. Compared to cv. Yulin, cv. Xiuyan was more tolerant to water stress-induced oxidative damage as evidenced by its relatively higher water status, CAT activity, and SOD activity. Moreover, relatively higher levels of free proline and lower levels of MDA in cv. Xiuyan were also associated with its better protection against water stress-induced oxidative damage. Results of Ali *et al.* (2010) on *Kochia* showed significant difference ($p < 0.05$) in CAT activity between control and stress treatments but there were no significant differences in CAT activity between control and recovered plants. CAT activity in stressed plants, increased by 97% compared with control. Birjand ecotype had more CAT activity (34%) compared with Borujerd. Recovered plants after drought stress statistically had CAT activity similar to control. Drought stress significantly ($p \leq 0.01$) increased APOX activity in *Kochia* leaves at flowering stage. APX activity increased 91% in stress treatment compared with control. Recovered plant did not show any significant effect in APOX activity compared with control. APX activity in Birjand ecotype was higher (59%) than that in Borujerd ecotype at stress treatment. The GR activity in the leaves of *Kochia* at stress treatment was 40% higher than that in control. There was no difference in GR activity between the ecotypes. However, GR activity in Birjand ecotype was 31% higher than that in Borujerd under stress conditions. POX activity didn't show any significant difference between treatment and ecotypes. SOD activity showed different patterns as compared to the other enzymes and recovered plants had the most SOD activity.

Devi *et al.* (2011) performed experiments on (*Triticum aestivum* L.) genotypes, each with different genetic background under normal and water deficit conditions induced

by 6% mannitol. In comparison with non-stressed seedlings, the catalase (CAT) activity was upregulated by more than 50% in the roots of water –stressed seedlings in drought–tolerant genotypes. Water deficit stress also led to the upregulation of ascorbate peroxidase (APX) in the endosperms and glutathione reductase (GR), CAT and POX in the shoots of stressed seedlings in the drought-tolerant genotypes. Superoxide dismutase activity was very low in the roots and shoots and showed non-significant increase under water-stress in tolerant genotypes. Out of the five specified enzyme activities (CAT in the roots and shoots, APX in the endosperm, GR and POX in shoots), if any three are up-regulated in the specified tissues under water deficit conditions, the genotype is likely to be drought-tolerant. Wheat seedlings with low GR and APX activities and high POX activity in shoots with low ratio of GR activity of shoot to root of non-stressed seedlings are likely to perform better under rainfed conditions.

In an experiment performed by Sharifi *et al.* (2012), changes in catalase and peroxidase activities in two levels of drought stress conditions (drought stress and control) and its effect on cell membrane and chlorophyll stability in the tolerant (Unknown 11, Homa and Ohadi), semi-tolerant (Sabalan and Rasad) and sensitive (SARAPBWYT-85-86-22-5 and SHARK-4-0YC-0YC-0YC-5YC-0YC) lines of wheat were analyzed in a factorial experiment based on randomized complete blocks with three replicates. The drought stress caused increase in the peroxidase enzyme activity of Unknown 11, Ohadi and Rasad lines. Although, no increase in peroxidase enzyme activity was shown, Homa still had the highest rate of enzyme activity in drought and control conditions. The activity of catalase enzyme in stress condition in all investigated lines remained stable or decreased and there was no specific relation between the activity of the enzyme and drought resistant. The highest index of chlorophyll stability in the stress condition was perceived in lines Homa and Ohadi and Unknown 11. Also, the most stabilized and the most inconstant cell membrane in stress condition was related to lines Rasad, Homa and SARA-PBWYT-85-86-22-5. In drought condition, there was a positive correlation between peroxidase enzyme activity, yield and stability of chlorophyll b, whereas we did not perceive any positive correlation between catalase enzyme activity and yield. Moreover, considering the existence of a negative correlation between peroxidase enzyme activity and cell membrane stability, authors concluded that more activity of peroxidase enzyme in

drought stress condition leads to increased cell membrane and chlorophyll stability, and it is related to the drought resistance of different lines.

Photosynthetic pigments, ascorbic acid and free amino acids content in leaves of *Malus domestica* Borkh. cv. 'Elstar' and cv. 'Jonagold wilmuta' subjected to mild drought or no drought were measured by HPLC in order to detect drought stress by Sircelj *et al.* (1999). Water deficit approximately (-1.9 MPa) increased ascorbic acid and zeaxanthin and decreased total chlorophyll (a+b), neoxanthin and β -carotene in leaves of cv. 'Jonagold wilmuta'. The results indicate that mild drought induced oxidative stress in leaves of cv. 'Jonagold wilmuta'. Drought of approximately -2.0 MPa) had no statistically significant effects on oxidative stress indicators measured in leaves of cv. 'Elstar'. The free amino acid content increased in leaves of stressed plants in both cultivars. The most pronounced difference was found between control and stressed leaves in Orn, Arg and Pro content in cv. 'Elstar' and Pro and Arg content in cv. 'Jonagold wilmuta'. The results indicate that the tolerance to water deficit depends on the apple tree cultivar

Mutants with decreased nonenzymic antioxidant contents have been shown to be hypersensitive to stress (Gao and Zang 2008; Semchuk *et al.* 2009). Studies done by Hura *et al.* (2008) showed that when maize plants were exposed to drought stress, the level of phenolic compound i.e., ferulic acid was decreased significantly due to the breakdown of the chlorophylls and phenolic pigments with the increase in drought intensity.

Ahmed *et al.* (2008) performed experiments in cotton leaves and their studies revealed that drought conditions reduced phenolic contents in cotton leaves at boll formation due to increased leaf senescence and degeneration of photosynthetic pigments.

Total phenolic contents in maize showed a decrease with increasing drought stress. This decrease in phenolic contents may be due to the degradation of photosynthetic pigments under drought stress (Ali *et al.* 2010). Gallagher *et al.* (2010) hypothesized that light limitation and drought during wild oat (*Avena fatua* L.) seed development would result in reduced allocation to seed phenolics and other aliphatic organic acids previously identified in the seeds of this species. Wild oat isolines (M73 and SH430) were grown in the greenhouse under cyclic drought conditions (2005 only) or two

levels of shade (50 and 70%; 2005 and 2006) achieved with standard black shade cloth. The soluble and cellular bound chemical constituents were identified and quantified using gas chromatography – mass spectrometry. The shade and drought stress treatments often significantly affected the mass of the caryopsis and hull seed fractions, as well as the phenolic content of these seed fractions, depending upon isolate, seed fraction, phenolic fraction, and specific phenolics analyzed. Phenolic content of the hull was reduced by the stress environments by up to 48%, whereas there was some evidence of an increase in the soluble phenolic content of the caryopsis in response to the stress environments. Ferulic and *p*-coumaric acids were the most abundant phenolic acids in both soluble and bound fractions, and bound phenolics comprised generally 95% or more of total phenolics.

Studies done by Kumar *et al.* (2011) gives insight into the relationship between oxidative damages caused by drought stress and variations in the level of antioxidants as part of the plants' protective mechanism in three rice genotypes IRBB21, Pusa Basmati 1 (PB1) and *O. longistaminata*. Augmentation in antioxidants, predominantly flavanoids, phenolics and total antioxidants were many folds higher in IRBB21 over PB1 and *O. longistaminata* during drought stress. The drought tolerant genotype *O. longistaminata* might have accumulated smaller amounts of free radicals and hence it produced lesser amount of antioxidant. The novelty of present work is that IRBB21 (resistant to biotic stress i.e., Bacterial blight (BB)) has produced more antioxidant, phenolics and flavanoids compared to Pusa Basmati1 (susceptible to BB) and *O. longistaminata* (tolerant to drought) to dehydration stress. Such observation suggests that increased water deficit induces a severe oxidative stress in rice plants, which apparently lead to enhance the antioxidant defense.

Ascorbate (AsA) is the most abundant, low molecular weight antioxidant that has a key role in defense against oxidative stress caused by enhanced level of ROS. AsA is considered powerful antioxidant because of its ability to donate electrons in a number of enzymatic and nonenzymatic reactions. AsA has been shown to play important role in several physiological processes in plants, including growth, differentiation, and metabolism. The majority of the AsA pool in plants is contributed by D-mannose/L-galactose commonly called Smirnoff-Wheeler pathway which proceeds via GDP-D-mannose, GDP-L-galactose, L-galactose, and L-galactono-1, 4-lactone (El-tayeb *et al.* 2007). AsA is also synthesized via uronic acid intermediates, such as D-

galacturonic acid (Li *et al.* 2010). In experiments performed by Ghorbanli *et al.* 2012 in Akria and Mobil cultivars of tomato, leaf ascorbate was increased in mild and severe drought stresses and the amount of ascorbate was higher in severe drought stress.

Tocopherols (α , β , γ , and δ) represent a group of lipophilic antioxidants involved in scavenging of oxygen free radicals, lipid peroxy radicals, and $^1\text{O}_2$ (Diplock *et al.* 1989). Relative antioxidant activity of the tocopherol isomers *in vivo* is $\alpha > \beta > \gamma > \delta$ which is due to the methylation pattern and the amount of methyl groups attached to the phenolic ring of the polar head structure (Fukuzawa *et al.* 1982). Hence, α -tocopherol with its three methyl substituents has the highest antioxidant activity of tocopherols (Kamal-Eldin and Appelqvist 1996). Carotenoids also belong to the group of lipophilic antioxidants and are able to detoxify various forms of ROS (Young 1991). Carotenoids are found in plants as well as microorganisms. In plants, carotenoids absorb light in the region between 400 and 550 nm of the visible spectrum and pass the captured energy to the Chl (Sieferman-Harms 1987). As an antioxidant, they scavenge $^1\text{O}_2$ to inhibit oxidative damage and quench triplet sensitizer (3Chl^*) and excited chlorophyll (Chl^*) molecule to prevent the formation of $^1\text{O}_2$ to protect the photosynthetic apparatus. Carotenoids also serve as precursors to signaling molecules that influence plant development and biotic/abiotic stress responses (Li *et al.* 2008). The ability of carotenoids to scavenge, prevent or minimize the production of triplet chlorophyll may be accounted for by their chemical specificity. Carotenoids contain a chain of isoprene residues bearing numerous conjugated double bonds which allows easy energy uptake from excited molecules and dissipation of excess energy as heat (Mittler 2002). Gomathi and Rakkiyapan (2011) observed that high carotenoids content favors better adaptation of sugarcane plants under saline condition. Carotenoids are one of the major non-enzymatic anti-oxidants that have a role in defense against water stress by scavenging of singlet oxygen and suppressing lipid peroxidation in all photosynthetic organisms. Carotenoids a lipid soluble antioxidant plays a multitude of functions in plant metabolism including oxidative stress tolerance (Gill and Tuteja 2010).

Experiments were performed by Terzi and Kadioglu (2006) to see the effect of drought stress on *Ctenanthe setosa* (Rosc.) Eichler during leaf rolling in the leaf, petiole. Chlorophyll and carotenoid content stability index decreased in the early

period of drought stress. Stability index decreased up to day 40 of drought and increased later, approximately reaching the control level on day 64. Under severe drought stress (25% of FC), total chlorophyll content in the cuttings belonging to the wet climate population decreased significantly, while there was no statistically significant differences between drought and well-watered treatment in the dry climate population during the whole experiment. Under moderate drought (55% and 40% of FC), the carotenoid content increased significantly in the stressed leaves for both populations but under severe drought (25% of FC), this value decreased in the wet climate population

Pandey *et al.* (2012) performed experiments in *Avena* species to determine the effect of drought stress at vegetative and flowering stage. At vegetative stage all the seven species of *Avena* grouped under three categories in response to the extent of moisture stress. The group one comprised of three species viz. *A. brevis*, *A. marocana* and *A. sterilis* showing the maximum stress up to 16th day from the day with holding the water. The second group comprised of 3 species (*A. strigosa*, *A. vaviloviana* and *A. abyssinica*) showing tolerance up to 12th day and the third group in which the stress tolerance goes up to 9th day includes only one species i.e. *A. sativa*. In group one with highest stress tolerance, *A. sterilis* considered to be the highest drought tolerant species in terms of minimum decrease in chlorophyll and carotene content even subjected to longest stretch of drought spell, followed by *A. strigosa* in group two at vegetative stage. Flowering stage six species showed tolerant to drought up to 5 days whereas *A. sterilis* showed extent of drought tolerance up to 4 days. There was less decrease in chlorophyll and carotene accumulation in *A. marocana* followed by *A. abyssinica* under water stress at flowering stage of crop growth therefore considered to be drought tolerant species during flowering stage.

2.1.4. Biomolecules

Accumulation of proline is a protective mechanism in a number of potential osmotic stresses, including salinity (Aghaei *et al.* 2009) water logging (Reggiani *et al.* 2000) and drought (Kim *et al.* 2004; Lee *et al.* 2009; Alam *et al.* 2010).

Proline and quaternary ammonium compounds, e.g. glycinebetaine, choline, prolinebetaine are key osmolytes contributing towards osmotic adjustment (Huang *et*

al. 2000 and Kavikishore *et al.* 2005). Accumulation of free proline is a common response in plants exposed to drought stress. (De Ronde *et al.* 2004; Hmida-Sayari *et al.* 2005; Kavikishore *et al.* 2005; Ashraf and Foolad 2007) It could function as hydroxyl radical scavenger to protect against membrane damage and protein denaturation. Several reports have demonstrated that proline accumulation in plants can enhance tolerance to abiotic stresses (De Ronde *et al.* 2004; Hmida-Sayari *et al.* 2005), and therefore proline accumulation can serve as the tolerance of most species to stress conditions (Ashraf and Foolad 2007).

In studies done by Koşkeroglu and Tuna (2010) proline content was increased in the leaves of maize plants grown at high salinity and water stress compared to the unstressed control plants. The proline content increased in drought-stressed plants and then decreased during the period of recovery in studies done by Alam *et al.* (2010).

Study was carried out by Najaphy *et al.* (2010) to investigate proline accumulation and protein profiles of leaves in three chickpea cultivars under normal watering (I1: irrigation based on 70 mm evaporation from class A pan), progressive water deficit (I2 and I3: 70...90...110...130 and 70...100...130 mm evaporation, respectively) and severe water stress (I4: 130 mm evaporation). The experiment was split-plot, based on randomized complete block design (RCBD) in three replications. By increasing irrigation intervals, leaf proline content increased. Probable stress responsive proteins in relation to imposed water deficit stress were determined by SDS-PAGE method. Water deficit stress increased concentration of soluble proteins in the leaves up to 43% in comparison with normal watering treatment (I1: 70...70), but did not significantly affect electrophoretic pattern of protein profiles. It seems that chickpea can be adapted to progressive water deficit stress conditions.

In order to assess drought tolerance mechanism in cotton Parida *et al.* (2007) performed experiments where, short-term drought-induced biochemical responses were monitored in two cotton (*Gossypium hirsutum* L.) genotypes contrasting their tolerance to water deficit. The seeds of two genotypes, namely GM 090304 (moderately drought tolerant) and Ca/H 631 (drought sensitive). The control plants showed a slight overall increase in total chlorophyll and carotenoid contents from 52 to 59 days after sowing. Total chlorophyll and carotenoid contents decreased significantly by drought induction in both the genotypes as compared to their

respective controls. On re-watering, both chlorophyll and carotenoid contents of the stressed plants tended to increase. The total chlorophyll expressed on unit dry weight basis decreased by 21% and 23% in GM 090304 and Ca/ H 631 genotypes, respectively, after 7 days of drought induction. When the drought-stressed plants were re-watered, a 32% increase in chlorophyll content of GM 090304 and 28% increase in Ca/H 631 were observed in recovered plants as compared to the drought-stressed plants. Similarly, after 7 days of drought induction, a 31% decrease in carotenoid content in GM 090304 and a 33% decrease in Ca/H 631 was observed as compared to their respective controls. Upon recovery from drought stress, carotenoids content increased significantly in recovered plants of both the genotypes (39% in GM 090304 and 34% in Ca/H 631). Chl a/b ratio of drought stressed plants decreased significantly in both the genotypes. However, Chl a/b ratio of both the genotypes recovered to control values after 7 days of rehydration.

Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Both the chlorophyll a and b are prone to soil dehydration (Farooq *et al.* 2009). Mohammadkhani & Heidari (2008); Efeoglu *et al.* (2009) found out that when maize plants were subjected to PEG induced drought stress their chlorophyll a, b and total chlorophyll contents were significantly decreased due to leaf senescence acceleration. Efeoglu *et al.* (2009) showed that when maize plants were subjected to PEG induced drought stress their chlorophyll a, b and total chlorophyll contents were significantly decreased due to leaf senescence acceleration.

Keyvan (2010) performed experiments on bread wheat cultivars and observed that drought stress had a significant effect on the content of chlorophyll a and b, and on the total of chlorophyll. It had a significant effect on the rate chlorophyll a to b. Content of chlorophyll a decreased because of drought stress and decrease of chlorophyll a, was lowest in Chamran cultivar and highest in Shahryar cultivar. Various cultivars were different significantly in different levels of drought stress so that the lowest decrease of chlorophyll a concern to Chanran cultivar and control

treatment and the highest decrease related to Shahryar cultivar and treatment. As the same of chlorophyll a, content of chlorophyll b decreased under effect of drought stress. In the light of chlorophyll b rate, various cultivars had a different reaction to drought stress, so that, the highest decrease of chlorophyll b was in Shahryar cultivar and the lowest decrease of chlorophyll b was in Chamran cultivar. Drought stress decreased the total rate of chlorophyll (the sum of chlorophyll a and b), too. The highest decrease of the total of chlorophyll, ratio to control treatment, belonged to treatment. In this case, various cultivars had different reactions, so that the highest rate of decrease of the total of chlorophyll (the sum of chlorophyll a and b) was in Shahryar cultivar and the lowest rate of decrease belonged to Chamran cultivar.

Masoumi *et al.* (2011) also observed that total chlorophyll content was altered by intensification in water deficit stress and decreased significantly in all of cultivars of *Glycine max* cultivars. Drought stress significantly decreased pigments content, but increased the ratio of carotenoids to total chlorophylls in two shrubs, *Pyracantha fortuneana* and *Rosa cymosa*, and four trees, *Broussonetia papyrifera*, *Cinnamomum bodinieri*, *Platycarya longipes* and *Pteroceltis tatarinowii*, were randomly assigned to four drought treatments in experiments performed by Liu *et al.* (2011).

Water stress also increases the levels of soluble sugars (compatible solutes) as glucose, sucrose, sorbitol, galactose, raffinose, stachyose and soluble proteins while it reduces the amounts of polysaccharides, starch and total sugars (El Tayeb 2006). Mohammadkhani and Heidari (2008) applied stress on maize cultivars with polyethylene glycol (PEG) 6000 and water potentials were: zero (control), -0.15 (PEG 10%), -0.49 (PEG 20%), -1.03 (PEG 30%) and -1.76 (PEG40%) MPa. The solutes accumulation of two maize (*Zea mays L.*) cultivars -704 and 301- were determined after drought stress. A higher amount of soluble sugars and a lower amount of starch were found under stress. Soluble sugars concentration increased (from 1.18 to 1.90 times) in roots and shoots of both varieties when the studied varieties were subjected to drought stress, but starch content were significantly ($p < 0.05$) decreased (from 16 to 84%) in both varieties. This suggests that sugars play an important role in Osmotic Adjustment (OA) in maize. Soluble sugar content and decrease of starch content in roots was higher than shoots.

An experiment was carried out by Keyvan (2010) to study the effects of drought stress on yield, proline content, soluble carbohydrates content, relative water content and chlorophyll content of bread wheat cultivars under field conditions. The results of variance analysis represented that changes of flag leaf soluble carbohydrates amount under the effect of cultivar and drought stress and from a statistical point of view were so significant. The mean comparison of flag leaf soluble carbohydrates amount in different wheat cultivars represented that Chamran cultivar and Marvdasht cultivar had the most amount of soluble carbohydrates and Shahryar cultivar had the lowest amount. Also, the mean comparison of flag leaf soluble carbohydrates amount in various treatments of drought stress represented that the treatment of drought stress had the most amount.

Experiments were performed by Naser *et al.* (2010) on Persian walnut. Plants exposed to water stress had a higher amount of soluble sugars in roots and shoots of tolerant genotypes ('Panegine20' and 'Chandler') and a lower amount of starch in their tissues. These results imply the important roles of soluble sugars as solutes conferring resistance to drought in these genotypes. The free proline levels were also increased in response to drought stress. They were higher in drought-tolerant genotypes than in sensitive ones ('Lara' and 'Serr'). Proline increased more in shoots than in roots. However, the soluble sugar and starch fluctuations were higher in the roots.

2.2. Influence of flooding on plant metabolism

Flooding stress is a wide spread phenomenon in regions where the soil has an impermeable clay base or consists of cracking grey clays with slow drainage, where there is an extreme rainfall pattern, or where imperfect land planning has occurred. Higher plants are aerobic organisms that die when oxygen availability is due to soil flooding (Voesenek *et al.* 2006). In recent years, many countries in the world have paid great attention to prevention of water logging, fighting against flooding, water and soil conservation, etc (Mielke *et al.* 2005). The soil water content of surface layer whenever exceeds at least 20% higher than field's carrying capacity it leads to free standing water on soil surface (Aggarwal *et al.* 2006). This condition imposes severe constraints on crop growth and productivity (Jackson and Colmer 2005). Soil flooding are major factors threatening the sustainability of agricultural land and natural ecosystems (Smedema and Shiati 2002). Water logging stress may develop due to

several direct (improper irrigation practices) and indirect (global warming) anthropogenic and natural consequences (meteorological) leading to altered plant metabolism, architecture and ecogeographical distribution depending upon plant responses. Alarming change in earth's average temperature (IPPC 2001) is predicted to exaggerate the problem in near future. The erratic rainfall, rise in sea level due to increasingly melting glaciers and shift in native climate-spectrum will pose selection pressure on agricultural crops. Therefore, highly resistant plants mingled with desired agronomical traits will be the demand of future crops. Rice, the global staple crop feeding above the half of the globe, has secured its growth in different climatic regimes accompanied by genotype selection. Flooding stress is a widespread phenomenon in regions where the soil has an impermeable clay base or consists of cracking grey clays with slow drainage, where there is an extreme rainfall pattern or where imperfect land planning has occurred. Higher plants are aerobic organisms that die when oxygen availability is limited due to soil flooding (Voesenek *et al.* 2006). It is one of the major abiotic constraints on agricultural productivity (Jackson and Colmer 2005) and has a devastating impact on crop growth and survival and thus on food production (Normile 2008). In Brazil, large agricultural constraints by flooding are found in flood plains areas (alluvial and hydromorphic soils), where temporary flooding restrict most agricultural crops, with the exception of flooded rice (Silva *et al.* 2007). Stress imposed by flooding is a major obstacle to growth, distribution, and productivity of plants. Natural factors, such as superabundance in precipitation, marginal water coming from rivers and bad drainage, influences the increment in flooding area (Sairam *et al.* 2008). The excess of water in the soil leads to dramatic consequences in the diffusive process (Vitorino *et al.* 2001; Ferreira *et al.* 2008), as the gases diffuse faster in air than in water and, therefore, there is a depletion of oxygen availability (hypoxia) both on the ground as in parts of the plant. The decline of oxygen is further accentuated by the presence of aerobic microorganisms that use this same gas in their processes in the rhizosphere, which may lead to extreme eradication of oxygen (anoxia) (Dat *et al.* 2004).

Land plants are strictly aerobes receiving freely diffusing molecular oxygen from aerial even underground tissues. Oxygen deficiency (hypoxia) or its complete absence (anoxia) damages most often the roots and seeds under transient or sustained flooded soil in various countries (Maltby 1991). Excess water produces anoxic soil within a

few hours (Gambrell and Patrick 1978). The slow diffusion rate (10–4 fold), low solubility (Ponnamperuma 1984; Gambrell *et al.* 1991) and increased microbial activity not only reduce the soil oxygen content, but also latter adds the toxic organic and inorganic compounds in the soil (Ponnamperuma 1984; Gambrell *et al.* 1991). The condition deteriorates during high temperature and in night, when root respiration increases with simultaneous increase of microbial activities. This rapidly depletes oxygen from soil rendering it with reduced inorganic forms of Fe^{3+} , S^{2-} and carboxylic acids, etc. Redox sensing of O_2 induces various biochemical, and therefore physiological changes in roots and shoots of several dicots, such as, in *Lycopersicon esculentum* (Jackson and Campbell 1976; Bradford and Yang 1981; Bradford and Hsiao 1982), *Solanum tuberosum*, legumes, *Nicotiana tobaccum*, *Ipomoea batatas*, *Cucumis sativus*, and monocots as in *Oryza sativa* (Vartapetian *et al.* 2003; Perata *et al.* 1992), *Triticum aestivum*, *Z. mays* (Saglio *et al.* 1988), and *Arabidopsis thaliana* (Trevaskis *et al.* 1997; Wang *et al.* 2000). A short spell of flood, however, may spare some moderately tolerant crops with the induction/up-regulation of counteracting genes to reverse the damages accompanied, at the cost of de-repression of certain other genes required for growth acceleration. A slow diffusion rate of O_2 under water pushes the plant (especially roots) towards more anaerobic biochemical acclimation (Armstrong 1978). Thus plants shift their metabolism at basal metabolic rate (BMR) to sustain their survival under low energy economy. Moreover, stagnate flooded water in lowland with added high precipitation climates may lead to sustained partial or complete water logging (submergence) may lethally push plant metabolism beyond BMR, where it may die ultimately. Therefore, the duration of water logging and genotype has critical relevance in stress defense. The mass destruction of crops causes a large reduction of global productivity every year. Reduced supply of adenylate pool set back the plant metabolism to anaerobiosis, by finely modulating the tissue based biochemical set up. However, rice employs this strategy at its initial level of seed germination avoiding hypoxia by extending coleoptile to reconcile with air (Jackson and Pearse 1991) and transporting oxygen from aerial part to hypoxic/anoxic roots indicating spatial and temporal pattern of the two strategies (true tolerance and avoidance, respectively) balancing under different growth and climatic conditions. Upsurge in massive literature in recent past regarding the defense strategies elicited by rice under flooding and related phenomena is indicative that rice has been at the

center of researches. Since water logging is soon followed by hypoxia, rice switches towards anaerobic metabolism particularly in roots. This condition hastens the aerobic mode of energy production with decline in adenylate pool (Irfan *et al.* 2010).

Oxygen deficiency affects the intensity and direction of a number of physiological and biochemical processes in the plant cells, inducing oxidative stress (Yordanova *et al.* 2004; Yordanova and Popova 2007) and also to morpho-anatomical changes in root and leaves (Pereira *et al.* 2008; Souza *et al.* 2009, 2010) affecting the nutrient and water uptake, so the plants show wilting even when surrounded by excess of water (Sairam *et al.* 2008). Lack of oxygen shift the energy metabolism from aerobic mode to anaerobic mode. Plants adapted to waterlogged conditions, have mechanisms to cope with this stress such as increased availability of soluble sugars, greater activity of glycolytic pathway and fermentation enzymes and involvement of antioxidant defense mechanism to cope with the post hypoxia/anoxia oxidative stress. Then the tricarboxylic acid cycle is interrupted by lack of reducing power and anaerobic metabolism is activated (Sairam *et al.* 2008). Production of ethanol and lactic acid though well-known fermentation pathways is one mechanism that organisms use to provide glycolytic substrate oxidation and ATP synthesis, maintaining short term cell viability under hypoxic conditions. ATP synthesis required to maintain plant growth and viability in an aerobic environment is achieved by the oxidation of carbon sources using oxygen as an electron acceptor. Under conditions that limit oxygen availability complete substrate oxidation is restricted by the lack of an electron acceptor. Two pathways are put forth yet so far operating under hypoxia, (1) anaerobic fermentation pathways and (2) alternative pathways, e.g., Hb–NO cycle (Sairam *et al.* 2009).

Plants susceptible to flooding have reduced photosynthesis (Chen *et al.* 2005) due to non-stomatal factors (biochemical) (Yordanova *et al.* 2005) and stomatal factors (Liao and Lin 2001). The tolerant plants, however, may be able to maintain its photosynthetic rate greater in addition to greater efficiency in the use of water, even in hypoxia (Li *et al.* 2004; Islam *et al.* 2008). Maize is one of the most important cereals cultivated in the world, since it presents high potential for grain production, nutritional value, and chemical composition. The inability of maize plants to endure low oxygen availability in the rhizosphere, caused by root flooding, result in substantial losses in productivity for example in South Asia, 15% of maize acreage is affected by flooding, whereas in India, water excess is already the second major

stress, reaching around 8.5 million hectares (Zaidi *et al.* 2007a). Positive attempts in search tolerant maize genotypes have been carried out through genetic selection, resulting in the development of plants with high yields and stable in water excess conditions (Zaidi *et al.* 2007b). Maize genotype tolerant to intermittent flooding have been reported, in these studies it was found that chlorophyll content, shoot dry weight, root volume, root porosity, grain yield, leaf area, and the interval between male and female flowering are quite contrasting in comparison with control (Lizaso *et al.* 2001; Zaidi *et al.* 2003, 2004). Gaseous plant hormone ethylene plays an important role in modifying plant response to oxygen deficiency. It has been reported to induce genes of enzymes associated with aerenchyma formation, glycolysis and fermentation pathway. Besides, non symbiotic-haemoglobins and nitric oxide have also been suggested as an alternative to fermentation (Sairam *et al.* 2008). Flooding stress made the root system turn brown or even black, repressed the generation of primary roots and promoted the growth of adventitious roots (Jing *et al.* 2001). Comparative analyses between flooding-tolerant and flooding-intolerant plants suggested that alteration of carbon and energy metabolisms during flooding or hypoxia were key responses in flooding-tolerant plants (Jackson and Ram 2003; Kreuzwieser *et al.* 2009). The effects of soil flooding on the physiological status of plants have been investigated in numerous papers (Yordanova and Popova 2007). Plants are either intolerant to flooding and therefore are excluded from flood-prone habitats, or they are tolerant. The latter group can be divided into plants that: exploit a so-called escape strategy based on a suite of (inducible) morphological and anatomical traits allowing re-aeration of flooded tissues; or those that adopt a quiescence strategy composed of traits that conserve the use of energy and carbohydrates to prolong underwater survival (Colmer & Voesenek 2009). When flood water deepens sufficiently to inundate the shoots as well as the roots, stress on the plants is much magnified. The extra stress arises because influx of aerial carbon dioxide for photosynthesis is largely prevented. Only a relatively small group of well- adapted aquatic or amphibious species can survive total submergence of the shoot system for long at growing temperatures. The principal strategy for survival is to shorten the period of total submergence by means of strong increase in the shoot elongation rate that reunites the shoot with air. In most cases this growth requires oxygen is regulated by a build-up of the plant hormone ethylene and is mediated via expression of expansion genes

(Voeselek *et al.* 2003; Vriezen *et al.* 2003). One of the negative consequences of soil flooding is oxygen deficiency in the tissues of the submerged organs of the plants. Oxygen deficiency affects the intensity and direction of a number of physiological and biochemical processes in the plant cells, inducing in this way oxidative stress (Yordanova *et al.* 2004; Yordanova and Popova 2007).

2.2.1. Morphological effects and relative water content

The most obvious changes after flooding are leaf chlorosis, necrosis, defoliation, cessation of growth, and premature plant death (Huynh *et al.* 2005). Studies on responses to flooding stress in soybean seedling have demonstrated that flooding reduces plant growth, especially root elongation, and inhibits hypocotyl pigmentation (Shi *et al.* 2008; Hashiguchi *et al.* 2009).

A greenhouse experiment was conducted by Ezin *et al.* (2010) to investigate the effect of flooding on two tomato cultivars and two wild related species. Forty days old tomato plants were subjected to a continuous flooding stress of different durations: 0, 2, 4 and 8 days. There was decrease in plant height of LA1421 genotype as the days of flooding increased however this was not statistically significant. In contrast there was slight increase in plant height of CLN2498E genotype for 4, and 8 day of continuous flooding, although this was not statistically significant. Significant differences were observed in the plant height of CA4 and LA1579 genotypes between the control and the flooded plants for 8 days of continuous flooding. 19 days after the continuous 8 flooding days was initiated until their death, plant height of LA1579 genotype significantly differed when compared to the control. Growth of LA1579 genotype seemed to be more negatively affected by flooding. The effect of flooding treatment (15cm above soil surface) on leaf number, and leaf length of CA4 and CLN2498E cultivars was not significantly different to their respective control. The number of leaves, however, slightly increased as the days of flooding increased with CLN2498E cultivar whereas it decreased slightly with CA4 cultivar due to the changes in plant height. Leaf length, in both cultivars, decreased slightly with the increase in days of continuous flooding. From 10 days after the flooding was initiated, significant differences in leaf number of both LA1421 and LA1579 wild related species were observed. Thus LA1579 genotype showed high degree of negative response to flooding compared to LA1421. Leaf length significantly differed between

flooded LA1421, LA1579 plants and their respective controls when treated with continuous 8 days of flooding for LA1421 genotype, and with continuous 4 and 8 days of flooding for LA1579 genotype. LA1579 genotype had the largest reduction in leaf length due to flooding conditions.

Celik and Turhan (2011) studied the tolerance to flooding stress in root regions of some common bean genotypes in terms of morphological and physiological parameters. Plants were grown in a plant growth chamber at 26/18°C (day/night) temperature with RH 70% and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity. Seedlings were exposed to flooding stress for 3 days when the plants had developed 3 to 4 true leaves. Effect of flooding treatment on RWC content of five common bean genotypes summarized that RWC of leaves was maintained despite the flooding in five common bean genotypes.

In experiments performed by Jahan *et al.* (2013) in order to see the effects of Water Stress on Rice Production. Relative water content in leaves of rice plants was measured in week 3, 6 and 9. The RWC in leaves was significantly higher in week 6 and 9 than that of RWC in leaves of plants grown in week 3 but data of RWC in either week showed insignificant difference. Their result also suggests that soil water condition at above saturation level, regardless of water depth, did not affect RWC in leaves.

2.2.2. Membrane damage and electrolyte leakage

In order to investigate the effects of spermidine (Spd) and spermine (Spm) on the water logging stress in (*Allium fistulosum* L.) Yiu *et al.* (2009) performed experiments were in stressed plants treated with Spd or Spm, the dynamics of water content decrease were found to be the same as those in untreated ones. In plants subjected to flooding stress, the rate of O_2^- generation increased by 240.4% and H_2O_2 content by 289.8% as compared with CK plants. F+Spd reduced the increased rate of O_2^- generation by 115.1% and H_2O_2 content by 140.5%, whereas F+Spm caused decrease of 84.6% and 91.6%, respectively. Exogenous Spd or Spm treatment without flooding stress also reduced O_2^- generation, with decrease of 26.01% and 21.79%, respectively, and H_2O_2 content, which decreased by 23.08% and 13.78%, respectively. After drainage of stressed plants, the contents of two estimated ROS were observed to drop. These results indicate that exogenous Spd and Spm can reduce the rate of O_2^- generation and the H_2O_2 content, and that the effect of Spd is better than that of Spm.

Lipid peroxidation of membranes can be estimated from the MDA content. In flooded plants, a rapid increase in MDA content was observed at day 1, and after 10 days of water logging, MDA levels were significantly higher than those found in the CK plants. Without stress, exogenous PA application did not significantly alter the MDA content. Exogenous application of Spd or Spm decreased the content of MDA by 95.04% and 39.64%, respectively, after 10 days of flooding treatment and by 128.3% and 39.07%, respectively, after 5 days of drainage. These results indicate that exogenous Spd and Spm treatment caused a significant decrease in the content of MDA under waterlogging treatment, and that the effect of Spd is better than that of Spm.

In the experiment performed by Balakhnina *et al.* (2009) to 13-27 days soil flooding and to 14 days of drainage following 13-days of soil flooding were studied on *Vicia faba*. The concentration of TBARs in the leaves of control plants showed a typical increasing tendency from 1.7 to 2.6 $\mu\text{mol g}^{-1}$ dry mass during 27 days of plant growth under experimental conditions. Under soil flooding the TBARs concentration in plant leaves increased strongly during first 2 days of flooding, reaching 3.2 $\mu\text{mol g}^{-1}$ dry mass. The elevated level of TBARs, 3.2–2.7 $\mu\text{mol g}^{-1}$ dry mass, which was significantly higher than that in control plants, maintained till the 13th day of the flooding experiment. After that the TBARs concentration in flooded plants dropped down and became significantly lower than in the control plants. Drainage of the soil caused a significant re-activation of the oxidative processes in the leaves; the TBARs concentration here was intermediate between the control and flooding treatments.

2.1.3. Antioxidative enzymes and antioxidants

Protection of plants from oxidative damage is associated with functioning of low molecular antioxidants (carotenoids, ascorbic acid, glutathione etc.) and antioxidant enzymes such as SOD, catalase, the enzymes of ascorbate-glutathione cycle and other pathways neutralizing ROS (Asada 2006). Excessive generation of reactive oxygen species (ROS), or oxidative stress, is an integral part of many stress situations, including hypoxia. Post-hypoxic hydrogen peroxide accumulation has been shown in the roots and in wheat roots (Biemelt *et al.* 2000). The presence of H_2O_2 in the apoplast and in association with the plasma membrane has been visualized by

transmission electron microscopy under hypoxic conditions in four plant species (Blokhina *et al.* 2001). ROS produced during hypoxia (*e.g.* H₂O₂) serve signal for the induction of antioxidant enzymes (Agarwal *et al.* 2005).

Pigeonpea plants suffered oxidative stress during water logging, which increased with duration of exposure, the increase in the activity of antioxidant enzymes during water logging is required to scavenge not only the post hypoxic ROS build up, but also to detoxify cellular system of ROS produced during hypoxia itself. Various antioxidative enzymes like SOD, APX, GR and CAT under waterlogged condition in tolerant and susceptible pigeon pea genotypes reveal a continuous increase (Kumutha *et al.* 2009).

The adaptive reactions of *Vicia faba* to 13-27 days soil flooding was studied by Tamara *et al.* (2010) to 14 days of drainage following 13-days of soil flooding were studied. Under flooding, oxygen diffusion rate (ODR) in the root zone decreased from 2.28–3.44 to 0.09–0.28 $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$; the soil redox potential (Eh) decreased from 543 to 70 mV. Upon drainage of flooded soil the ODR and Eh values returned to the control levels. Oxidative damage and defense systems in leaves were assessed by the concentration of thiobarbituric acid reactive substances (TBARs) and by the activities of SOD and GR. Two stages of stress development are described. During the first stage (1–13 days) shoot dry mass did not decrease, the TBARs concentration and SOD activity increased, the GR activity decreased. The second stage (13–27 days) was characterized by a decrease in the TBARs concentration, SOD and GR activities, pigment concentrations and shoot dry mass. Drainage of flooded soil resulted in elevated concentrations of TBARs and also increased the activities of SOD and GR. Increased SOD activity in the first stage of hypoxic stress development and activations of SOD and GR at oxygen re-entry to soil are responsible for tolerance of *Vicia faba* to hypoxic and post hypoxic stress associated with soil flooding and subsequent drainage.

Lin *et al.* (2006) studied the changes to the antioxidative system in the leaves of three sweet potato varieties, Taoyuan 2, Simon 1, and Sushu 18 as affected by flooding and drought stresses. The experimental design was completely randomized with a split plot arrangement of treatments. Young, fully expanded leaves from each plant were clipped for antioxidant activity measurement. They concluded that genotypes exhibited their abilities and specificities on porphyrins, polyphenol, flavonoids,

reduction power and scavenging DPPH radical and superoxide anion. The polyphenol content and scavenging superoxide anion percentage of the three sweet potato varieties under the stresses declined significantly. However, the conjugated dienes inhibition percentage increased markedly under the stresses. Inhibiting the conjugated dienes could mitigate flooding and drought stress effects and be useful as a flooding and drought-tolerant index.

In experiments performed by Tamara *et al.* (2010) in *Vicia fabia* in flooding stress showed that the concentration of carotenoids in the leaves of control plants did not show a tendency to change with time. Carotenoid concentration in the leaves of flooded plants, as compared with the control ones, decreased significantly after 15 days of soil flooding.

2.2.4. Biomolecules

Plants adapted to waterlogged conditions, have mechanisms to cope with this stress such as increased availability of soluble sugars, greater activity of glycolytic pathway and fermentation enzymes and involvement of antioxidant defense mechanism to cope with the post hypoxia/anoxia oxidative stress (Sairam *et al.* 2008).

Yiu *et al.* (2009) performed experiments on flooded Welsh onion plants (*Allium fistulosum* L.) to investigate the effects of spermidine (Spd) and spermine (Spm) on the waterlogging stress. Plants untreated with PAs experienced decreases in root proline content, whereas only with Spm application was there no significant difference with respect to CK values throughout the experimental period. F+Spd and F+Spm reached proline levels 2.6- and 2.0-fold higher than those of flooded plants after 10 days of continuous water logging, and the levels remained high until the end of the experimental period. After drainage, proline levels decreased to values 1.5- and 1.1-fold higher than flooded plants at day 15.

In studies done by Tamara *et al.* (2010) on oxidative damage and antioxidant defense system in leaves of *Vicia faba*, the adaptive reactions of *Vicia faba* was studied to 13-27 days soil flooding and to 14 days of drainage following 13-days of soil flooding. Chlorophyll (a+b) concentration in the leaves of control plants did not change considerably during 27 days of growth. Soil flooding caused a significant decrease in this parameter after 15 days of the experiment; at the end of the experiment (27 days) chlorophyll concentration in the leaves of flooded plants was

63% from that in the control ones. Drainage of the soil did not succeed to increase significantly the shoot biomass and chlorophyll concentration above that of the flood treatment. Experiments were performed by González *et al.* (2009), on Quinoa (*Chenopodium quinoa* Willd.) to study how the plants respond to drought and water logging. Total chlorophyll and chlorophyll a and b content was lower in plants under water logging than in drought and control, and contents of the latter two showed no significant differences. Leaf total soluble sugars, sucrose, glucose, fructose, and starch contents were higher in water logging conditions than in drought or control treatments. However, drought showed higher values of total soluble sugars and glucose than control while fructose, sucrose and starch contents showed no significant differences. Leaf soluble protein content was also higher under water logging than in drought or control conditions. In addition, the lowest value ($70.6 \text{ mg g}^{-1} \text{ DW}$) was observed in control leaves. With respect to proline content the highest value ($0.47 \text{ mg g}^{-1} \text{ DW}$) was observed under drought while water logging and control treatments revealed no significant differences.

2.3. Amelioration of water stress by chemical pre-treatments

In the study done by Somasundaram *et al.* (2009), paclobutrazol, a triazole growth regulator and abscisic acid (ABA), a traditional growth regulator were used to analyse their role in water stress amelioration in drought stressed *Sesamum indicum* L. plants. The main aspects studied were the antioxidant enzyme profile changes in different parts of treated, drought stressed as well as control plants. The enzymes superoxide dismutase, ascorbate peroxidase, and peroxidase, were analyzed. It was found that, these growth regulators can be used as stress ameliorating agents in this crop plant, as they increase the antioxidant enzyme activities and there by providing stress tolerance ability to the plant.

The effect of drought stress and ABA applied at tillering stage (55 days after sowing) was compared in 2 wheat cultivars differing in drought tolerance by Bano *et al.* (2012). The activities of superoxide dismutase (SOD) and peroxidase (POD) and contents of endogenous ABA in plants were measured at 3 days of drought stress in cv. Chakwal-97 (drought tolerant) and cv. Punjab-96 (drought susceptible). ABA was applied at 10^{-6} mol/L as pre sowing seed treatment for 18 h. Drought tolerant cultivar has a more efficient mechanism to scavenge reactive oxygen species as shown by a

significant increase in the activity of antioxidant enzyme SOD. Under drought stress, ABA significantly increased the activities of SOD and POD, showing a significant decline on rewatering. The relative water content was significantly increased by ABA priming under drought stress in both wheat cultivars. The sensitive cultivar exhibiting lower endogenous ABA content was more responsive to ABA priming. On rewatering, the magnitude of recovery from drought stress was greater in tolerant cultivar. ABA was highly effective in improving grain weight of tolerant cultivar under drought stress.

A study was carried out by Iqbal *et al.* (2005) to assess whether exogenously applied glycinebetaine has any role in reducing the adverse effects of water stress on sunflower achene yield and oil contents. Two sunflower lines, Gulshan-98 and Suncross were subjected to water stress at the vegetative and reproductive stages of plant growth. Three levels of glycinebetaine (0, 50 and 100 mM) were applied before sowing (seed treatment) and at the time of initiation of stress at the vegetative and reproductive stages. A marked adverse effect of water stress on 100-achene weight and achene oil contents were observed in both sunflower lines. Exogenous supply of glycinebetaine was not effective in alleviating the adverse effects of water stress on achene oil percentage. Foliar spray of glycinebetaine, however, significantly reduced the negative effects of water stress on achene weight. Seed treatment with either level of glycinebetaine was not effective in increasing the 100- achene weight and achene oil percentage under both normally irrigated and water stress conditions. The sunflower line, Suncross produced higher oil yield than that of Gulshan-98.

Another study was carried out by Ali *et al.* (2013) to appraise whether or not the exogenous application of a potential osmoprotectant, proline, could ameliorate the adverse effects of drought stress on maize seed and seed oil composition, as well as oil antioxidant activity. Water stress reduced the kernel sugar, oil, protein and moisture contents and most of the seed macro- and micro-elements analyzed in both maize cultivars but it increased the contents of seed fiber and ash. Water stress increased the oil oleic acid content with a subsequent decrease in the amount of linoleic acid, resulting in an increased oil oleic/linoleic ratio for both maize cultivars. However, no variation was observed in oil stearic and palmitic acids content due to water stress. A considerable drought induced an increase in seed oil α -, γ -, δ - and total tocopherols and flavonoids were observed in both maize cultivars. However, oil

phenolic and carotenoid content as well as 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity decreased. Foliar-applied proline significantly increased the content of seed sugar, oil, protein, moisture, fiber and ash in both maize cultivars under well irrigated and water deficit conditions. Furthermore, exogenous application of proline increased the oil oleic and linoleic acid contents. The concentrations of antioxidant compounds namely phenolics, carotenoids, flavonoids and tocopherols estimated in the seed oil increased due to foliar-applied proline under water deficit conditions that was positively correlated with the enhanced oil DPPH free radical scavenging activity. Moreover, the increase in the contents of these antioxidant compounds and oil antioxidant activity due to the foliar application of proline was noted to be more pronounced under water deficit conditions.

With the aim to contribute to elucidation of the role of phytohormones in plant responses to stresses the endogenous contents of abscisic acid (ABA) and cytokinins (CK) were followed in French bean, maize, sugar beet, and tobacco during water stress and subsequent rehydration by Pospíšilová *et al.* (2005). The effects of pre-treatments with exogenous ABA or benzyladenine (BA) before imposition of water stress were also evaluated. The content of ABA increased by water stress, and with the exception of bean plants increased content of ABA remained also after rehydration. In all plant species the ABA content was further increased by ABA pre-treatment, but in bean and maize it decreased by BA pre-treatment. The highest total content of CK was observed in bean and the lowest in maize during water stress. In their spectrum, the storage CK was dominant in bean and inactive CK in tobacco while in sugar beet and maize all groups were present in comparable amounts. In all plant species, the contents of CK increased during water stress and with exception of bean they decreased back after rehydration. ABA pre-treatment further increased contents of CK in water-stressed bean and tobacco. BA pretreatment increased contents of CK in sugar beet and tobacco after rehydration.

Study to find out the response of wheat (*Triticum aestivum* L.) cultivars (Lasani-2008, Auqab-2000) to foliar application of 1 % potassium at different growth stages (tillering, flower initiation and milking) was carried out under water limited environment, at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad during 2008-09 by Aown *et al.* (2012). The wire house experiment was laid out in completely randomized design. Data regarding various agronomic traits (plant height,

spike length, number of spikelets per spike, number of grains per spike, 1000-grain weight and grain yield per plant) of crop were recorded using standard procedures. The data so collected were analyzed statistically by using the Fisher's analysis of variance technique and LSD at 5% probability was used to compare the differences among treatments' means. Drought stress at all three critical growth stages adversely affected plant height, spike length, number of spikelets per spike, number of grains per spike, 1000-grain weight and grain yield of wheat plant. Foliar application of K at all three critical growth stages improved the drought tolerance of plants and improved the growth and yield components, however, grain filling stage was found more responsive.

Experiments were performed by Manivannan *et al.* (2007) to see the ameliorating effect of calcium chloride on sodium chloride-stressed plants of *Vigna radiata* (L.) Wilczek. Plants were treated with solutions of 100 mM NaCl, 100 mM NaCl with 5 mM CaCl₂, or 5mM CaCl₂. Groundwater was used for irrigation as the control. Plants were harvested randomly 30 and 50 days after sowing. NaCl and CaCl₂-stressed plants showed reduced growth as indicated by decreased root length, stemlength, total leaf area and dry weight. Proline and glycine betaine content and the activity of the antioxidant enzymes superoxide dismutase, ascorbate peroxidase and catalase were increased under treatment with NaCl alone and CaCl₂ alone. When CaCl₂ was combined with NaCl, CaCl₂ altered the overall plant metabolism to ameliorate the deleterious effects of NaCl stress and increased the vegetative growth of the plants.

Arginine (precursor of polyamine, i.e., spermidine, spermine, putrescine) is considered one of exogenous protectant which may alleviate the harmful effects of salinity stress. To achieve the aforementioned objective, two pot experiments were performed by Hozaynat *et al.* (2013), at the screen greenhouse of Agronomy Department, National Research Centre, Cairo, Egypt to study the effect of pre-soaking of mung bean seeds in different concentrations of arginine (0.0, 0.3, 0.6 and 1.2 mM) on growth, yield and chemical composition of yielded seeds of mung bean plants grown under different salinity levels (1500, 3000, 4500 and 6000 ppm) in 2008 and 2009 summer seasons. All growth parameters (plant height and dry weight) were significantly reduced at the highest salinity levels (3000, 4500 and 6000 ppm), while 1500 ppm induced slight increase. Salinity induced significant increases in Na and significantly decreased N, P, and K contents in green leaves of mung bean, as well as,

yield components (plant height, pods number and weight, seeds number/pod, seeds weight/plant and biological yield/plant) and nutritional value (soluble carbohydrate, polysaccharides, total carbohydrate, proline, total amino acids and total protein) of seeds were reduced. Pre-treating mung bean seeds with arginine with different concentration 0.3 and 0.6 and 1.2 mM exhibited significant increments in mung bean growth, yield and yield components and nutritional value of yielded seeds compared to the untreated plants. The magnitude of increments was much more pronounced in response to 0.3 mM of arginine. From their study it could be concluded that, pre-soaking seeds of mung bean with arginine could alleviate the harmful effect of salinity especially at the lower levels of salinity of at all the studied parameters.

The effects of potassium fertilizer (K_2SO_4) levels K_0 , (0), K_1 , (150) and K_2 , (250 kg/ha) in two species of *Brassica napus* (Hyola 401 Hybrid) and *Brassica juncea* (landrace cultivar), under three irrigation regimes, control (irrigation after 50%), moderate stress, (irrigation after 70%), and severe stress (irrigation after 90% soil water depletion) were studied in a factorial experiment laid out in a randomized complete block design with three replications by Fanaei *et al.* (2009). Grain yield and physiological indices, including relative water content (RWC), stomatal conductance (g), chlorophyll content (SPAD values); leaf temperature (TL), and the difference between canopy temperature and air temperature ($T_c - T_a$) were measured at two stages (50% flowering and 100% siliques formation). Both species maintained, higher RWC, SPAD values and g, in non stress condition, but decreasing soil water supply caused a lower RWC, SPAD values, g, Δt and increased TL. Potassium application also improved above mentioned physiological traits. Grain yield was positively associated with RWC, g and SPAD values but showed a negative association with TL and Δt in both stages. Results showed that with increasing stress severity grain yield reduced significantly, but potassium application conferred great increase on rapeseed yield. Overall, grain yield showed significant association with RWC, g, SPAD values, Δt and TL under this experiment conditions. It is concluded that potassium application, could ameliorate negative effects of water stress on grain yield and physiological properties and consequently improved them. For selecting drought tolerant cultivars, due to easier measurement of g, SPAD values and TL, they could be recommended for screening large numbers of rapeseed cultivars in a short time at critical stages of crop growth.

Experiments were performed by Umar (2006) to study the alleviating adverse effects of water stress on yield of sorghum, mustard and groundnut by potassium application. Plants were subjected to increased soil water stress conditions, tissue K concentrations analysed at peak growth stage (flowering) and relationships worked out between tissue K concentrations, yield parameters and relative water content. The water content of the leaf tissue was significantly increased by K application and the highest increase in RWC was 14.7 %, 17.4 % and 22.8 % under normal conditions, and by 8.7 %, 19.9 % and 17.7 % under water stress conditions in mustard, sorghum and groundnut, respectively. Water stress caused grain yield reductions and K application could enhance yield to a great extent. Production of above ground biomass, grain yield and RWC were highly correlated with the tissue K concentration, showing that concentration of K⁺ in leaves played a vital role in increasing water stress resistance and stabilizing yield in the crops studied.

3.1. Plant material

Seven cultivars of maize (*Zea mays* L.) seeds –Bharat Nath 101 (BN-101), Bharat Nath 1133 (BN-1133), Dhanya 849 (DMH-849), Swarna, Pioneer 30V92, Kaveri Super 244(KS-244) and Kaveri Super 244+ (KS-244+) were selected for experimental purpose. These cultivars were obtained from a seed center from Siliguri town, West-Bengal.

3.2. Growth and maintenance of the plant

The seeds were surface sterilized with 0.1% (w/v) HgCl_2 , where the seeds were first washed with HgCl_2 for 3-4 minutes and then washed thrice with sterile distilled water each time keeping the water for 3 minutes. Then the thoroughly washed seeds were transferred into autoclaved petriplates in aseptic condition. The seeds were allowed to germinate in a Seed Germinator at a temperature of 20°C , RH 65% and 16h photoperiod. For one set of treatment the one week old seedling were transferred to earthen pots sized 12" height and 9" diameter containing soil mixed with farmyard manure in the proportion of 2:1 ratio; another set of plants were transferred in small thermocoile cups containing soil mixed with farmyard manure in the proportion of 2:1 ratio. Plants were allowed to grow in the glass house which maintained a temperature of $20\text{-}25^\circ\text{C}$, RH 65-70% and 16h photoperiod. Plants were watered regularly twice daily-morning and evening and weeding was done once a week, till the time of treatment. Another set of plants were maintained in the Seed Germinator for *in vitro* experiments in the petriplates itself and Hoagland solution was given to the seedlings till the time of treatment.

3.2.1. Preparation of Hoagland solution

Hoagland's solution for application in seedlings which were kept for In vitro experiment was prepared by the process given by Gamborg and Wetter (1975).

Solution A: Boric Acid (H_3BO_3)-0.280g, $\text{MnSO}_4\cdot\text{H}_2\text{O}$ -0.340g, $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ -0.010g, $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ -0.022g and $(\text{NH}_4)_6\text{MO}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ -0.010g was dissolved in 50mL of deionized water and then the volume was made up to 100mL and stored at 4°C for further use.

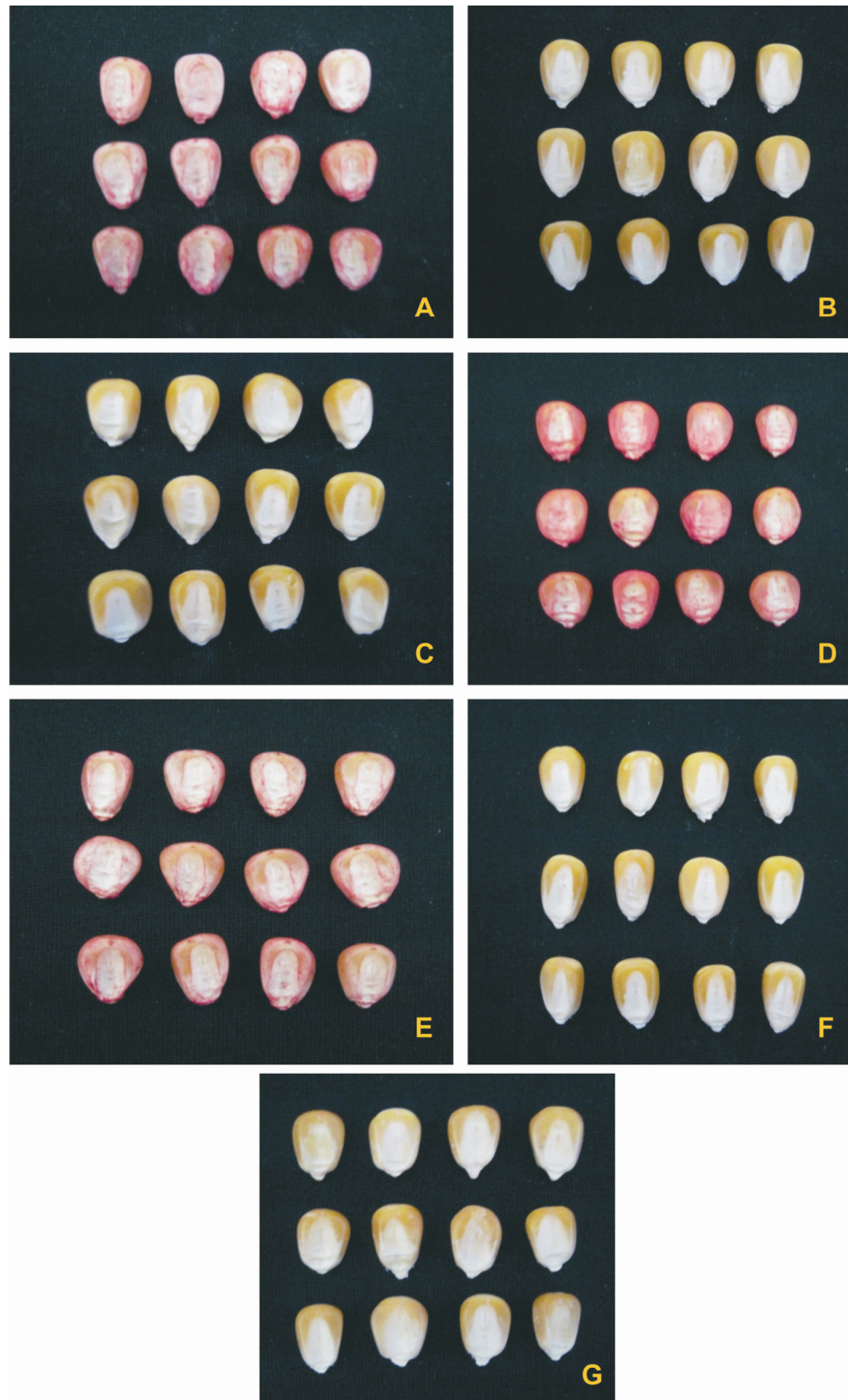


Fig 1: Seeds of seven different cultivars of maize (*Zea mays* L.)
A: KS 244 **B:** BN101 **C:** BN1133 **D:** KS 244+
E: SWARNA **F:** DHANYA **G:** 30V92

Solution B: 0.5mL of conc.H₂SO₄ was added to 80mL of deionized water and then the total volume was made up to 100mL and stored at 4⁰C for further use.

Solution C: Na₂EDTA-3.36g and FeSO₄- 2.79g was dissolved in 200mL of deionized water, then the solution was at a temperature of 70⁰C with continues stirring until the colour of the solution turned yellow-brown. Then the solution was cooled and volume was adjusted to 500mL and stored at 4⁰C for further use.

Hoagland's Stock Solution (10X): Ca(NO₃)₂.4H₂O-2.35g, MgSO₄.7H₂O-1.3g, KNO₃-3.3g and NH₄H₂PO₄- 0.6g was dissolved in 200mL of deionized water after which 5mL of solution A and 0.5mL of solution B was added to it and the total volume was adjusted to 500mL with deionized water and stored at 4⁰C for further use.

Hoagland's Nutrient Solution (1X): 100mL of 10X stock solution was mixed with 5mL solution C and then the volume was adjusted to 1000mL deionized H₂O. This solution was prepared just before use.

3.3. Treatments

3.3.1. Plants subjected to drought stress

3.3.1.1. Induction of water stress *in vivo*

Two month old plants of all the seven test cultivars that were grown in the glass house was subjected to drought stress by completely withholding water in the pots of the test plants, and then the sampling of the plants were performed on the 3rd, 5th, and 7th day of drought stress along with the sampling of the well watered control plants.

3.3.1.2. Induction of water stress *in vitro*

One week old seedlings of all the seven cultivars was subjected to drought stress by applying 10% (w/v) PEG-6000 (Polyethylene glycol), and sampling was done on the 3rd, 5th and 7th day of stress along with the sampling of the control plants which was done on 0-day.

3.3.1.3. Induction of drought stress in two cultivars of maize in the field

One week old seedlings of two cultivars KS 244 and Dhanya (DMH 849) was transferred to a plot in the field and was watered daily till the time of flowering, then the plants were subjected to drought stress by withholding water and drought

subjected plants were sampled on the 20th day of drought stress along with the control which was sampled on 0-day of stress.

3.3.2. Induction of flooding stress

One set of two week old plant of all the seven cultivars of maize that was grown in the earthen pots was subjected to flooding stress by holding water in the pots. The test plants were then sampled on the 3rd, 5th and 7th day of stress. Control plants were sampled on the 0-day of stress.

3.4. Determination of Relative water content (RWC)

Fresh weight 0.500g of leaf discs were taken initially and then the leaf discs was immersed in 10ml of double distilled water for 24hrs at room temperature. After 24hrs they were taken out from the water and excess water on the surface was removed using blotting paper and then was weighed to determine the turgid weight. After taking the weight the leaf discs were dried at 80°C for 24hrs and then the dry weight was taken. Relative water content (RWC) of leaves was determined as described by Farooqui *et al.* (2000). The following formula was used to calculate the RWC (%): $(\text{Fresh weight} - \text{Dry weight}) / (\text{Fully turgid weight} - \text{Dry weight}) \times 100$.

3.5. Determination of Lipid peroxidation

Lipid peroxidation was measured as MDA determined by the Thiobarbituric acid (TBA) reaction. Cells (0.250g) were homogenized in 2 ml of 0.1% (w/v) Trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 10 min. To 0.5 ml of the aliquot of the supernatant, 2ml of 20% TCA containing 0.5% (w/v) TBA were added. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. The absorbance was measured at 532 nm and 600. The concentration of MDA was calculated using an extinction coefficient of 155 mM⁻¹ given by Heath and Packer (1968).

3.6. Evaluation of cell membrane injury

Electrolytic leakage was measured by the process given by Lutts *et al.* (1996) with few modifications. 0.100g of plant material was made into leaf discs and was washed thoroughly with sterile distilled water, placed in tubes with 15ml of deionized water and incubated for 2h at 25°C after which the electrical conductivity was measured (L₁). Samples were then placed in boiling water at 100 °C for 20min and then the final

conductivity (L_2) was measured after equilibration at 25°C. Electrolytic leakage was recorded in a conductivity meter (Labindia) with $K=0.946$, cell constant=1, solution condition=84 μS , coefficient-1 at 25°C.

The EL was defined as follows:

$$\text{EL (\%)} = (L_1/L_2) \times 100$$

3.7. Tolerance index determination

Tolerance index of the leaves of plant sample was determined by the method given by Wilkins (1978) with minor modification. 1g of fresh leaf sample was placed in a drying cabinet at 80 °C for 12hr and finally weighed. Tolerance index (T_i) was calculated as: $T_i = \text{Dry weight of leaves of treated plants (DW}_T) / \text{Dry weight of leaves in control plants (DW}_C)$.

3.8. Extraction and estimation of carbohydrates

3.8.1. Extraction of total soluble sugar and reducing sugar

Total and reducing sugar was extracted by the method given by Harborne (1973). 0.250g of leaf and root tissues was extracted in 10mL 95% ethanol and the alcoholic fraction was vaporized on a boiling water bath in a beaker. The residue was re-extracted and the process was repeated. The aqueous fraction was re-dissolved in distilled water and made up to 5mL which was then centrifuged at 5000rpm for 10mins. The supernatant was collected and volume was made up to 5ml with distilled water.

3.8.2. Extraction of starch

Starch extraction from leaf and root was done by the method given by Thimmaiah (1999), where 0.250g of plant tissue was extracted in hot 80% ethanol. The homogenized mixture was filtered and extraction was done repeatedly for 3-4 times from the residue by washing the residue with hot 80% ethanol till the filtrate showed no reaction with Anthrone's reagent (0.2% Anthrone in H_2SO_4) and gave no colour. The residue was dried over a water bath and then 5mL of distilled water and 6.5mL of 52% perchloric acid was added to it. Then the extract was centrifuged at 0°C at 6000 rpm for 20mins. This process was carried out twice. The supernatant was pooled and the final volume was made up to 10mL by adding distilled water.

3.8.3. Estimation of total soluble sugar

Estimation of total sugar was done by the Anthrone's method given by Plummer (1978). In 1mL of test solution, 4mL of Anthrone's reagent was added (0.2% Anthrone in conc. H_2SO_4). The reaction mixture was mixed thoroughly and was incubated in boiling water for 10mins. Then the reaction mixture was cooled under running tap water and absorbance was measured in a colorimeter at a wavelength of 620nm against a proper blank and quantified by using a standard curve of glucose.

3.8.4. Estimation of reducing sugar

Reducing sugar was estimated by Somogyi-Nelsons method as described by Plummer (1978) in which 1mL of the test solution was mixed with 1mL of Alkaline copper tartarate solution (2g- CuSO_4 , 12g- Na_2CO_3 anhydrous, 8g- $\text{Na}^+\text{-K}^+$ tartarate, 90g- Na_2SO_4 anhydrous-in 500mL of distilled water) and heated over a boiling water bath for 20mins. Then after the reaction mixture was cooled under running tap water 1mL of commercially available Nelson's arseno molybdate reagent was added to the reaction mixture along with 2mL of distilled water and mixed vigorously. A blue colour was developed which was the measured in a colorimeter at 515nm against a proper blank and quantified using a standard curve of glucose.

3.8.5. Estimation of starch

Estimation of starch was done by the method given by Thimmaiah (1999). 1mL of test solution was mixed with 4mL of Anthrone's reagent (0.2% Anthrone in conc. H_2SO_4) and mixed thoroughly. The mixture was then placed in boiling water for 8mins. Then the reaction mixture was cooled under running tap water after which absorbance of the reaction mixture was measured at 630nm in a colorimeter against a proper blank. Starch content was then quantified by using a standard curve of starch.

3.9. Quantification of proline content

3.9.1. Extraction

Extraction of proline from the leaves and root was done by the method of Bates *et al.* (1973). 1g of plant tissue was homogenized in 10mL of 3% Sulfosalicylic acid and filtered through a Whatman No. 1 filter paper. The supernatant was collected for estimation.

3.9.2. Estimation

To 1mL of extract, 3mL of distilled water and 1mL of Ninhydrin solution (1g Ninhydrin + 10mL acetone + 15mL distilled water) was added. The reaction mixture was kept on a boiling water bath for 30mins and then cooled at room temperature. The reaction mixture was transferred in separating funnel and 5mL of Toluene was added and mixed vigorously. The lower coloured layer was taken and the OD values were measured at 520nm in a colorimeter against a blank and quantified from a standard curve of proline.

3.10. Extraction and estimation of chlorophyll

Chlorophyll was estimated according to the method of Harborne (1973) by homogenizing 0.250g of leaf tissue in 80% acetone and filtered through Whatman No.1 filter paper. 80% acetone was repeatedly added from the top till the residue became completely colourless. Then the filtrate was collected and total volume was measured. Estimation of chlorophyll was done by measuring the OD at 663nm and 645nm respectively in a UV-VIS spectrophotometer (UV-VIS Spectrophotometer 118 systronics) against a blank of 80% acetone and calculated using the formula as given by Arnon (1949).

$$\text{Total chlorophyll} = (20.2 A_{645} + 8.02 A_{663}) \text{ mg g}^{-1} \text{ fresh weight}$$

$$\text{Chlorophyll a} = (12.7 A_{663} - A_{645}) \text{ mg g}^{-1} \text{ fresh weight}$$

$$\text{Chlorophyll b} = (22.9 A_{645} - 4.68 A_{663}) \text{ mg g}^{-1} \text{ fresh weight}$$

3.11. Estimation and extraction of phenols

3.11.1. Extraction

The phenol content was extracted by the method given by Mahadevan and Sridhar (1982). 0.250g of leaf tissue was immersed in a beaker containing 5mL of boiling absolute alcohol in dark for 10 mins cooled and then crushed in motor pestle using 80% alcohol in dark and then filtered in a dark chamber. The residue was re-extracted with 80% alcohol and then final volume was made up to 10mL with 80% alcohol.

3.11.2. Estimation of total phenols

Total phenols from leaf tissue was estimated by the process given by Bray and Thorpe (1954). In 1mL of extract, 1mL of 50% (1:1) diluted Folin ciocalteu's phenol reagent

and 2mL of 20% Na_2CO_3 solution was added. The reaction mixture was boiled in water bath for 1min and the cooled under running tap water followed by dilution with distilled water and final volume was made up to 25mL. Then the absorbance was measured at 650nm in a colorimeter against a proper blank and quantified using a standard curve of ferulic acid.

3.11.3. Estimation of ortho-dihydroxy phenol

Estimation of ortho-dihydroxy phenol from leaf tissue was done by the method given by Arnows (1937). To 1mL of the extract, 2mL of 0.5(N) HCl, 1mL of Arnow's reagent (10g- NaNO_2 , 10g- Na_2MoO_4 in 100mL of distilled water) and 2mL of 1(N) NaOH was added. A pink colour was developed and volume was made up to 10mL with distilled water. After vigorous shaking the absorbance was measured at 515nm in a colorimeter against a proper blank and quantified using a standard curve of ferulic acid.

3.13. Extraction and Estimation of antioxidative enzymes

3.13.1. Extraction

For extraction of enzymes, mature leaf samples were initially ground to powder in liquid nitrogen and then extracted with 50 mM sodium phosphate buffer (peroxidase, catalase (pH 6.8) and ascorbate peroxidase (pH 7.2)) and 100 mM potassium phosphate buffer, pH-7.6 (glutathione reductase and total superoxide dismutase) using polyvinylpyrrolidone under ice cold conditions. The homogenates were then centrifuged at 10,000 rpm for 10 min at 4°C. Supernatants were used as crude enzyme extracts and estimation of protein content was done in each case following the method of Lowry (1951).

3.13.2. Estimation

3.13.2.1. Peroxidase (POX EC 1.11.17)

Peroxidase activity was assayed as described by Chakraborty *et al.* (1993) spectrophotometrically in UV-VIS spectrophotometer 118 systronics at 460 nm by monitoring the oxidation of O-dianisidine in the presence of H_2O_2 . The reaction mixture consisted of 1.7mL of distilled water, 1mL of 0.2M sodium phosphate buffer (pH 5.4), 0.1 mL of H_2O_2 , 0.1mL of freshly prepared Ortho-dianisidine (5mg/ml Ortho-dianisidine in methanol) and 0.1mL of enzyme extract. The change in absorbance was noted down immediately at an interval of 1-min for 4-minutes against

a blank. The assay specific activity was expressed as mmol O-dianisidine mg protein⁻¹ min⁻¹.

3.13.2.2. Catalase (CAT EC 1.11.1.6)

Catalase activity was assayed as described by Chance and Machly (1955) spectrophotometrically in UV-VIS spectrophotometer 118 systronics measured at 240nm estimating the breakdown of H₂O₂. 40μL of the enzyme extract was added to 3mL of H₂O₂ buffer (0.1mL of H₂O₂ in 25mL of NaPO₄ buffer of pH 6.8) and mixed well. The change in absorbance of the sample was noted down immediately after every 15-sec for 3-minutes. The enzyme activity was expressed as μmol H₂O₂ mg protein⁻¹ min⁻¹.

3.13.2.3. Ascorbate peroxidase (APOX EC.1.11.1.11)

Ascorbate peroxidase activity was assayed as described by the method of Asada and Takahashi (1987) by monitoring the oxidation of ascorbate at 290nm spectrophotometrically in UV-VIS spectrophotometer 118 systronics. The reaction mixture consisted of 0.01mL of freshly prepared Ascorbic acid (10mg ascorbic acid in 10mL of 0.05M sodium phosphate buffer (pH-7.2), 0.01mL of H₂O₂, 2.97mL of 0.05M of sodium phosphate buffer (pH 7.2) and 0.01mL of enzyme extract was added just before assay and the change in absorbance of the sample was measured immediately at an interval of 1-minute for 3-minutes. Enzyme activity was expressed as mmol ascorbate mg protein⁻¹ min⁻¹.

3.13.2.4. Superoxide dismutase (SOD EC 1.15.1.1)

Superoxide dismutase activity was assayed by the method of Dhindsa *et al.* (1981) by monitoring the inhibition of the photochemical reduction of Nitroblue tetrazolium at 560nm spectrophotometrically in UV-VIS spectrophotometer 118 systronics. The assay mixture contained 1.5mL of 0.1M potassium phosphate buffer (pH 7.6), 0.8mL of distilled water, 0.1mL of 1.5M Na₂CO₃, 0.1mL of freshly prepared 3mM EDTA (Ethylene diaminetetraacetic acid), 0.1mL of 2.25mM NBT (Nitroblue tetrazolium chloride), 0.2mL of 0.2M freshly prepared Methionine, 0.1mL of 60μM Riboflavin and 0.1mL of enzyme extract was added just before use. The reaction tubes containing enzyme samples were illuminated with 15W fluorescent lamp for 10min. Another set of tubes lacking enzymes were illuminated and served as control. A non-

irradiated complete reaction mixture served blank. The absorbance of the samples was measured at 560 nm and 1 unit of activity was defined as the amount of enzyme required to inhibit 50% of the Nitroblue tetrazolium reduction rate in the controls containing no enzymes.

3.13.2.5. Glutathione reductase (GR EC 1.6.4.2)

Glutathione reductase activity was assayed by the method of Lee and Lee (2000) by monitoring the oxidation of NADPH at 340 nm spectrophotometrically in UV-VIS spectrophotometer 118 systronics. The reaction mixture consisted of 1mL of 0.1M potassium buffer (pH-7.6), 0.2mL of 0.1M EDTA (Ethylene diaminetetraacetic acid), 0.1mL of freshly prepared 6mM Glutathione, 0.2 mL of 0.1mM NADPH (Nicotinamide adenine dinucleotide phosphate reduced tetrazolium salt) and 0.2mL of enzyme extract. Change in absorbance was determined immediately at an interval of 1-minute for 3-minutes against a blank which contained the entire reaction mixture and extraction buffer instead of the enzyme extract. Enzyme activity was expressed as $\mu\text{mol NADPH oxidized mg protein}^{-1} \text{ min}^{-1}$.

3.14. Isozyme analysis by Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide gel electrophoresis was performed for isozyme analyses of different enzymes by following the method given by Davis (1964).

3.14.1. Preparation of stock solution

Solution A: Acrylamide stock solution for resolving gel

28g of acrylamide and 0.76g of N' N' Methelene bis-acrylamide was dissolved in 50mL of warm distilled water and then volume made up to 100mL for the preparation of solution (A). Then the solution was filtered through No.1 filter paper and stored at -4°C in a dark bottle for further use.

Solution B: Acrylamide stock solution for stacking gel

Solution (B) was made with 10g of acrylamide and 2.5g of N' N' Methelene bis-acrylamide which was dissolved in 50mL of warm distilled water and then the volume was made up to 100mL and filtered through No.1 filter paper and stored at -4°C in a dark bottle for further use.

Solution C: Tris-HCl (pH-8.9) for resolving gel

Tris-HCl was prepared by dissolving 9.15g of Tris in 15mL of double distilled water and the desired pH was maintained by adding 1(M) HCl to the solution after the pH was adjusted the volume of the solution was made up to 25mL with doubled distilled water and then stored at - 4°C for further use.

Solution D: Tris-Hcl (pH-6.7) for stacking gel

Tris-HCl was prepared by dissolving 1.495g of Tris in 15mL of double distilled water and the desired pH was maintained by adding 1(M) HCl to the solution after the pH was adjusted the volume of the solution was made up to 25mL with doubled distilled water and then stored at - 4°C for further use.

Solution E: Ammonium persulphate solution (APS)

Fresh solution of Ammonium persulphate was prepared by dissolving 0.15g of APS in 100 mL of distilled water.

Solution F: Riboflavin solution

A solution of Riboflavin (F) was made by dissolving 4mg of riboflavin in 10mL of distilled water and stored in dark brown bottles at - 4°C and stored for further use.

Gel loading dye was prepared by mixing 0.1g of Bromophenol blue and 4g of sucrose in 10mL of distilled water and filtered which was then stored at - 4°C for further use. Electrophoresis buffer was freshly prepared on the day of experiment by dissolving 0.18g of Tris and 0.87g of Glycine in 100mL of double distilled water and the final volume was made up to 300mL and stored at - 4°C.

3.14.2. Sample preparation

1g of plant leaf tissue was crushed in 5mL of sodium phosphate buffer (pH 7.2) in liquid nitrogen in a pre-chilled mortar and pestle, centrifuged at 10, 000 rpm in a cooling centrifuge at -4°C for 20min. The supernatant was stored in vials at - 4°C for further use.

3.14.3. Preparation of gel and electrophoresis

Gel was prepared by taking two mini slab glass gel plates (8cm×10cm) which was thoroughly cleaned in order to remove any traces of grease and then dried. 1mm thick spacers were placed between two glass plates (both sides and below) and sealed with

vacuum grease and clipped from all sides in order to prevent leakage of the gel solution leaving the top open. Resolving gel solution was prepared by mixing solution A, C, E and H₂O (distilled) the ratio 1:1:4:1, 5 μ L of TEMED (N,N,N',N'-Tetramethyl ethylenediamine) was added to it and gently mixed then immediately with the help of pasture pipette it is poured in the prepared gel plate leaving sufficient space for the stacking gel on top. Then the resolving gel was immediately over layered with water and kept for polymerization for 2hrs. After 2 hrs the over layer water was poured off and washed with distilled water to remove any unpolymerized acrylamide. Then the stacking gel solution was prepared mixing B, D, F and distilled water in the ratio of 2:1:1:4 along with 4 μ L of TEMED (N,N,N',N'-Tetramethyl ethylenediamine) and poured on top of the resolving gel immediately after which the comb was inserted, leaving a sufficient space between the resolving and stacking gel. Then again it is over layered with distilled water. The gel was then kept for polymerization for 1hr in strong sunlight. After polymerization of the stacking gel the comb was removed carefully and the wells were cleaned thoroughly with distilled water. After removing the clips and the lower spacer the gel plate was mounted in the electrophoresis apparatus. The Tris-Glycine running buffer that was prepared was then poured in the apparatus and was added sufficiently in both the upper and lower reservoir. Any bubbles trapped at the bottom of the gel were removed very carefully with a bent syringe. Then the electrodes were adjusted in their respective places. 35 μ L of the sample was mixed with 15 μ L of gel loading dye for loading it in the lanes. Then the mixed sample was loaded in each lane of the gel and separated for about 2-3hrs at 10mA current in ice-cold condition at - 4°C inside a refrigerator till the dye front reached the bottom of the gel.

3.14.4. Staining procedure

After electrophoresis the gel was removed from glass plate and the two plates were carefully separated, then the stacking gel was carefully removed and the resolving gel was then placed in the staining box and stained using suitable dye.

3.14.4.1. Peroxidase

Peroxidase isozyme was visualized by following the staining method of Reddy and Garber (1971) in ice-cold condition. For the staining purpose Benzidine dye was made where 0.52g of Benzidine was mixed in 4.5mL of Glacial acetic acid, after

thoroughly dissolving the benzidine in acetic acid, 20mL of chilled distilled water was added to it and filtered through Whatman no. 1 filter paper. 3% H_2O_2 was prepared separately in 25ml of chilled distilled water. The two solutions were mixed just before use and immediately poured on top of the resolving gel in a gel staining box. Dark blue coloured bands were observed after staining. The reaction was arrested by immersing the gel into a large volume of 7% acetic acid solution. Analysis of the bands for their R_m value was immediately done and photographs were taken for further analysis.

3.14.4.2. Catalase

Catalase isozyme was visualized by following the staining method of Woodbury *et al.* (1971) in ice-cold condition. The gel after electrophoresis was soaked in 3.3mM H_2O_2 for 20min in chilled condition. The gel was then rinsed thrice in chilled distilled water and was then incubated in freshly prepared 1% Potassium ferricyanide and 1% Ferric chloride in 1:1 ratio for again 20mins. Yellow bands in green background appeared on the gel whose R_m value was analysed and immediately photographs were taken for further analysis.

3.15. Extraction and quantification of non-enzymatic antioxidants

3.15.1. Ascorbate

Extraction and quantification of ascorbic acid from the leaves was done following the method described by Mukherjee and Choudhuri (1983). The leaf tissue was homogenised in pre-chilled mortar and pestle on ice using 6% Trichloroacetic acid and filtered. Total volume was made up to 10 mL. For estimation, to 4mL of the extract, 2mL of 2% Dinitrophenylhydrazine (in acidic medium) and 1 drop of 10% Thiourea (70% ethanol) was added. The reaction mixture was thoroughly shaken kept in boiling water for 15mins. and cooled at room temperature. Then 5mL of 80% (v/v) sulphuric acid (H_2SO_4) was added to the reaction mixture at 0°C . The absorbance was then taken at 530nm against a blank in UV-VIS spectrophotometer 118 systronics. The concentration was quantified using the standard curve of ascorbic acid.

3.15.2. Carotenoids

The extraction and quantification of carotenoids was done by following the method of Litchenthaler (1987). For extraction, 0.250g of leaf tissue was homogenized in 100%

methanol in dark condition and filtered in whatman filter paper 1. Total volume was made up to 10mL. Then absorbance was measured at 480nm, 645nm and 663nm against a blank in UV-VIS spectrophotometer 118 systronics and was calculated using standard formula:

$$A_{480} - (0.114 \times A_{663}) - 0.638 (A_{645}) \mu\text{M g}^{-1} \text{ fresh weight}$$

3.15.3. α -Tocopherol

Vitamin E (α -Tocopherol) was extracted and estimated by the process of Paquot (1982) with some modifications. Leaf tissues were homogenized 5mL of hexane and vigorously shaken. Then 2mL of the extract, 2 mL of absolute ethanol was added and mixed thoroughly, to which 2mL of 0.2mL of α ', α ' - Dipyrindyl solution (0.5% in ethanol) and 0.2mL of Ferric chloride solution (0.2% in ethanol) was added and shaken well. The reaction mixture was then incubated in dark for 15mins. After the development of red colour 4mL of distilled water was added to the above mixture and mixed well. Two layers were formed which was then separated out by a separating funnel and the lower red coloured aqueous layer was collected and the absorbance was measured against a blank at 520nm in UV-VIS spectrophotometer 118 systronics and quantified using a standard curve of α -tocopherol.

3.16. Determination of Hydrogen peroxide (H₂O₂) accumulation

3.16.1. Extraction and quantification of hydrogen peroxide

The extraction and estimation of hydrogen peroxide was done by the method given by Jena and Choudhuri (1981). The leaf extract was homogenized in 12mL of phosphate buffer (50mM, pH 6.5) in a dark chamber and centrifuged at 6000 rpm for 25mins. The supernatant was used for estimation where 3mL of extract was mixed with 1mL of 0.1% Titanium sulphate in 20%(v/v) H₂SO₄ along with a blank containing 1mL of crushing buffer instead of the extract. Both the blank and the reaction mixture was centrifuged again at 6000rpm for another 15mins. The intensity of yellow colour was then measured at 410nm in UV-VIS spectrophotometer 118 systronics and quantified using the extinction coefficient (0.28 $\mu\text{mol}^{-1} \text{ cm}^{-1}$).

3.16.2. Microscopic detection

The detection of hydrogen peroxide in the leaf tissue was done according to the method given by Thordal-Christensen *et al.* (1997) with minor modifications. The leaf

tissues were washed thoroughly with doubled distilled water and cut into leaf discs. Then these leaf discs were incubated in dark for 24h in 1mg/ml of 3, 3'-Diaminobenzidine (DAB) having pH-3.8. After 24h the leaf disc was transferred to a beaker containing 90% ethanol and was heated at a temperature of 70°C it was rinsed in ethanol until all the chlorophyll was removed. Photographs of the leaf discs were taken after it was observed under the microscope. Hydrogen peroxide was visualized as reddish-brown colour at the site of Diaminobenzidine polymerization.

3.17. Estimation of total antioxidant activity

The estimation of total antioxidant activity was done by the method of Blois (1958). 1g of dried leaf sample was ground into fine powder and was then extracted in 100% methanol and centrifuged at 4000rpm for 10mins. The supernatant was poured in a beaker and the methanol was evaporated off. Then the dried part was finally dissolved in 5mL of absolute methanol. The estimation was done by taking 80µL of the sample and 2mL 2, 2-Diphenyl-1-Picrylhydrazyl (0.0025g in 100mL methanol). Initial absorbance was taken in 0-min (AT_0) and then the reaction mixture was kept in dark and after 30-min (AT_{30}) another absorbance was taken at 517nm in UV-VIS spectrophotometer (Model 118 systronics). The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation:

$$\text{Inhibition (\%)} = (AT_0 - AT_{30}) / AT_0 \times 100.$$

3.18. Protein analysis

3.18.1. Extraction of soluble protein

Soluble protein from the leaves and root of maize samples were extracted by following Chakraborty *et al.* (1995). Fresh plant tissue was homogenized in a pre-chilled motor and pestle in ice using 5mL of 50mM sodium phosphate buffer (pH-7.2) and polyvinylpyrrolidone under ice cold condition and centrifuged at 10,000 rpm at -4°C for 15mins. The supernatant obtained was used as the crude extract for the estimation.

3.18.2. Quantification

Quantification of soluble protein was done by following the methodology of Lowry *et al.* (1951). To 1mL of crude extract of protein sample, 5mL of freshly prepared Alkaline reagent (2% Na_2CO_3 in 100 mL of 0.1N NaOH, to which 1mL each of 1%

CuSO₄ and 2% Na⁺ - K⁺ tartarate was added just before use) was added. The reaction mixture was incubated for 15mins after which 0.5mL of 1(N) Folin Ciocalteu's phenol reagent was added and again incubated for 20mins. Absorbance was measured in a spectrophotometer (UV-VIS spectrophotometer 118 systronics) at 690nm against a proper blank and quantified using a standard curve of Bovine serum albumin.

3.18.3. SDS-PAGE Analysis

SDS PAGE analysis for observing the protein pattern of the sample was done by the methodology given by Sambrook *et al.* (1989).

3.18.3.1. Preparation of stock solution

In order to prepare the gel for electrophoresis the following solutions are prepared

(A) 30% Acrylamide solution:

29% Acrylamide and 1% N' N'-Methylene bis-acrylamide (7.25 g of acrylamide and 0.25g of N' N'-Methylene bis-acrylamide) was mixed in 25mL of warm distilled water. Then the solution was filtered through Whatman No.1 filter paper, and kept in a dark bottle at 4⁰C for further use.

(B) SDS (Sodium Dodecyl Sulphate):

10% stock solution of SDS (1g SDS dissolved in 10mL of distilled water) was prepared in warm distilled water and stored at room temperature.

(C) Tris buffer:

(i) 1.5M Tris buffer for resolving gel was prepared by dissolving 4.542g of Tris base in 15mL of distilled water and the pH of the solution was adjusted to 8.8 using concentrated HCl. The final volume of the solution was made up to 25mL with distilled water and stored at - 4°C.

(ii) 1.0M Tris buffer for stacking gel was prepared by dissolving 3.029g Tris base in 15mL of distilled water and the pH of the solution was adjusted to 6.8 using concentrated HCl. The final volume of the solution was made up to 25mL with distilled water and stored at - 4°C.

(D) Ammonium Per sulphate (APS) solution:

Ammonium per sulphate solution was freshly with 10% APS dissolved in distilled water.

(E) Tris-Glycine electrophoresis running buffer:

Tris running buffer was freshly prepared by dissolving 25mM of Tris base and 250mM of Glycine in distilled water, and after mixing it thoroughly 0.1% of SDS was also mixed with the solution.

(F) SDS gel loading buffer

This buffer was made by mixing 50mM Tris-HCl (pH-6.8), 10mM β -Mercaptoethanol, 10% SDS, 0.1% Bromophenol blue and 10% Glycerol. Therefore a 1X solution in 10mL contained 1mL of 50mM Tris-HCl, 0.5mL of β -mercaptoethanol, 2mL of 10%SDS, 10mg of bromophenol blue, 1mL of glycerol, and the volume was made up to 10mL using distilled water and stored at - 4°C.

(G) Fixing solution

The fixing solution was prepared by mixing glacial acetic acid, methanol and distilled water in the ratio of 10:20:10 and was stored at normal room temperature.

(H) Staining solution

The staining solution was prepared by dissolving 250mg of Coomassie brilliant blue (Sigma R-250) in 45mL of methanol. After which 45mL of distilled water along with 10mL of glacial acetic acid was added to it. The prepared stain was filtered through Whatman No. 1 filter paper and stored at room temperature.

(I) Destaining solution

The destaining solution was prepared by mixing methanol, distilled water and acetic acid in the ratio of 45:45:10 and stored at room temperature.

(J) 7% acetic acid solution was prepared at the time of use for storing of the gel.

3.18.3.3. Preparation of gel and electrophoresis

Mini slab gel plates (8cm x 10cm) were used in SDS-PAGE gel electrophoresis. Gel was prepared by taking two mini slab glass gel plates (8cm x 10cm) which was thoroughly cleaned in order to remove any traces of grease and then dried. 1.5 mm

thick spacers were placed between two glass plates (both sides and below) and sealed with vacuum grease and clipped from all sides in order to prevent leakage of the gel solution leaving the top open.

In order to make resolving gel solution (10%) 2.85mL of distilled water, 2.55mL of 30% Acrylamide solution, 1.95mL of 1.5M Tris-buffer (pH-8.8), 0.075mL of 10% SDS, 0.075mL of 10% APS and 0.003mL of TEMED (N,N,N',N'-Tetramethylethylenediamine) was mixed together and then poured in the gel plate with the help of Pasture pipette leaving sufficient space for the stacking gel on top. The resolving gel was immediately over layered with water and kept for polymerization for 1hrs. After 1h the over layer water was poured off and washed with distilled water to remove any unpolymerized acrylamide. Then the stacking gel solution (5%) was prepared by mixing 2.10mL of distilled water, 0.500mL of 30% acrylamide solution, 0.380mL of 1.5M Tris buffer (pH-6.8), 0.030mL of 10% SDS, 0.030mL of 10% APS and 0.003mL of TEMED, which was poured on top of the resolving gel immediately after which the comb was inserted, leaving a sufficient space between the resolving and stacking gel. Then again it is over layered with distilled water. The gel was again kept for polymerization of the stacking gel for 1hr. After polymerization of the stacking gel the comb was removed carefully and the wells were cleaned thoroughly with distilled water. After removing the clips and the lower spacer the gel plate was mounted in the electrophoresis apparatus. The Tris-Glycine running buffer that was prepared was then poured in the apparatus and was added sufficiently in both the upper and lower reservoir. Any bubbles trapped at the bottom of the gel were removed very carefully with a bent syringe. Then the electrodes were adjusted in their respective places. 35 μ L of the sample was mixed with 15 μ L of gel loading dye for loading it in the lanes. Then the mixed sample was loaded in each lane of the gel and separated at 18mA current till the dye front reached the bottom of the gel.

3.18.3.2. Preparation of sample

The soluble proteins were extracted from the leaf tissue by the method given by Demirevska *et al.* (2008). 1g of leaf tissue was homogenized in 5mL of ice-cold 100mM Tris-HCl (pH-8) containing 20mM MgCl₂, 10mM NaHCO₃, 1mM EDTA (Disodium salt), 2mM PMSF (Phenylmethanesulfonyl fluoride), 12.5% Glycerol (v/v) and 20mM of β -Mercaptoethanol in a pre-chilled motor and pestle under ice-cold

condition and centrifuged at 10,000 rpm at - 4°C for 20-mins. The supernatant was used as crude extract for SDS-PAGE in order to analysis the protein patterns of the sample. 35µL of the crude sample was mixed with 15µL of 1X SDS gel loading dye buffer. Then the samples were placed in boiling water bath for 3-mins denature protein sample. They were then immediately loaded in the wells of the gel using a micropipette.

3.18.3.3. Fixing, staining and destaining

After electrophoresis was complete the gel plate was taken out from the apparatus and then the gel was carefully separated from the glass plate. The stacking layer was discarded and the remaining resolving gel portion was carefully transferred to a gel box of appropriate size and left overnight in 40mL of fixing solution. Then the next day, the fixing solution was properly decanted from the gel box after which. 40mL of staining solution was immediately poured on top of the gel and kept for about 4-6hrs on a shaker with a constant speed of 30rpm for proper staining. After the staining of the gel was done, the staining solution was decanted out from the gel box and destaining solution was added to it and kept on a shaker with 30 rpm until destaining was achieved. The destaining solution in the gel was repeatedly replaced with fresh destaining solution till the time the protein patterns were distinct. Then the gel was stored in 7% acetic acid solution for analysis. Analysis of the bands for their R_m value was done and photograph of the gel was also taken.

3.19. HPLC analysis of phenolics

3.19.1. Sample preparation

3.19.1.1. Total phenols

Extraction of phenols from the leaves of the sample for HPLC analysis was done by the method given by Pari & Latha (2004). 10g of fresh leaf tissues were cut into small pieces and soaked overnight in absolute methanol in dark. Next day the suspension was filtered and the filtrate was completely evaporated-off using a rotary evaporator and was again re-dissolved in 2mL of HPLC grade methanol and filtered through a 0.45µm filter.

3.19.1.2. Phenolic acid

Phenolic acids were separated by the method given by Amarowicz & Weidner (2001). with slight modification. 10g fresh leaf tissues were cut into small pieces and soaked overnight in absolute methanol in dark. Next day the suspension was filtered and the filtrate was completely evaporated-off using a rotary evaporator. The extract was then suspended in 10mL of 2 mol/ L NaOH and was kept for 4h for hydrolysis at normal room temperature in dark. Then the suspension was acidified by using 6mol/L HCl (pH-2). The free phenolic acids and constituents liberated from esters were then extracted 4 times using 15mL of diethyl ether in a separating funnel. Ether from the extraction was removed by evaporation until the extract was dried. The dry residue of free phenolic acids and phenolic acids which was liberated from esters was re-dissolved in 2mL of HPLC grade methanol and filtered through a 0.45µm filter.

3.19.2. Total phenol analyses

Analysis of total phenols in HPLC was done by the method given by Crozier *et al.* (1997). For the HPLC finger print analysis of phenolic compounds present in extracts a Shimadzu system (Shimadzu Corp., Kyoto, Japan) was used, a flow rate of 1 mL/min, and gradient elution of acetonitrile-water-acetic acid (5:93:2, v/v/v) [solvent A] and of acetonitrile-water-acetic acid (40:58:2, v/v/v) [solvent B], a 0– 50 min solvent B from 0 to 100%; and injection volume of 20 µL were applied; and the separation of compounds was monitored at 280nm.

3.19.3. Analyses of phenolic acids

Phenolic acids were analysed by the methodology given by Amarowicz & Weidner (2001) using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan). The mobile phase water-acetonitrile-acetic acid (88:10:2; v/v/v) was delivered at a rate of 1 mL/min and 20µL of injection volume was applied. The detection was monitored at 320 nm.

3.20. Application of chemicals

Two chemicals in different concentrations were applied to two cultivars of maize Dhanya and KS-244) in order to see the ameliorative effects of the chemical on the two cultivars during drought and flooding stress. This experiment was performed on one month old plant samples in the pot culture.

3.20.1. Absciscic acid

One month old plant samples of two cultivars of maize were sprayed with 50 μ M and 100 μ M of Absciscic acid (ABA) solution mixed with 0.1% of Tween 80, twice a day (early morning and late afternoon) in the form of foliar spray for 4 days, after which drought was induced in these foliar sprayed sets along with sets which was not sprayed with any chemicals of both the cultivars by withholding water for 7 days along with well watered controls.

3.20.2. Proline

One month old plant samples of two cultivars of maize were sprayed with 30mM and 60mM of proline solution mixed with 0.1% of Tween 80, twice a day (early morning and late afternoon) in the form of foliar spray for 4 days, after which drought was induced in these sets along with sets which were not sprayed with any chemical of both the cultivars by withholding water for 7 days along with well watered controls.

4.1. Effect of water stress on morphological characteristics and relative water content

Seven cultivars of maize were selected for study to determine differences in their responses to drought stress and flooding. Different sets of experiments were performed using these cultivars.

4.1.1. *In vivo* imposition of drought

In the first set two months old plant of all the seven cultivars were subjected to drought stress in soil (Fig 2 and Fig 3). These plants were subjected to 0-day, 3-day, 5-day and 7-day of drought stress by withholding water in the pots. All the seven cultivars showed no morphological changes in the initial period of stress. Slight wilting was seen from the 5th day of drought stress in all the cultivars. The results of relative water content from the leaves of these cultivars further made it stronger that the above mentioned cultivars were more susceptible to drought stress than the rest of the cultivars (Fig 4).

4.1.2. *In vivo* flooding

The second set of experiment was performed on all the seven cultivars of maize plant where the plants were subjected to flooding stress by holding water in the pots for 0-day, 3-day, 5-day and 7-day. Morphological changes were very much evident in case of Dhanya and Swarna after the 5th day of flooding (Fig 5 and Fig 6). The results of relative water content from the leaves of the seven cultivars showed that there was a maximum decrease in Dhanya and Swarna cultivar during flooding stress (Table 1).

4.1.3. Induction of drought *in vitro*

The third set of experiment was performed on all the seven cultivars, where the plants were subjected to 0-day, 3-day, 5-day and 7-day of drought stress *in vitro* by application of 10% (PEG-Polyethylene glycol). Morphological difference was very pronounced in Dhanya, Swarna and BN 1133 on the 7th day of drought stress (Fig 7). Relative water content from the leaves of seven cultivars also showed that there was a maximum decrease in RWC in Dhanya, Swarna and BN 1133 (Table 2).



Fig.2: Four cultivars of maize (*Zea mays* L.) subjected to drought stress *in vivo*



Fig.3: Three cultivars of maize (*Zea mays* L.) subjected to drought stress *in vivo*

Table 1: Relative water content from the leaves of maize cultivars subjected to flooding stress *in vivo*

CULTIVARS	Relative Water Content (%)			
	0d	3d	5d	7d
KS 244	96.43±0.526 ^a	91.44±2.199 ^a	82.70±0.499 ^b	74.68±0.382 ^b
KS 244+	93.97±0.639 ^a	93.77±0.427 ^a	88.07±0.802 ^b	79.82±0.619 ^b
BN 101	94.89±0.645 ^a	90.60±1.084 ^a	87.26±0.531 ^b	79.46±0.329 ^b
30V92	91.04±0.047 ^a	86.38±0.424 ^b	76.34±0.254 ^b	65.32±0.166 ^b
BN 1133	95.14±0.859 ^a	87.57±0.517 ^b	72.29±0.894 ^b	61.13±1.859 ^b
SWARNA	88.58±0.518 ^a	79.86±0.682 ^b	68.35±0.592 ^b	48.67±0.378 ^b
DHANYA	91.04±0.047 ^a	87.55±1.376 ^a	67.04±0.525 ^b	50.53±1.149 ^b

CD- Treatments: 5.84; Cultivars: 7.72 Average of three replicate experiments. $\pm$ =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 2: Relative water content from the leaves of maize cultivars subjected to drought stress *in vitro*

CULTIVARS	Relative Water Content (%)			
	0d	3d	5d	7d
KS 244	90.71±0.259 ^a	85.86±2.100 ^a	83.86±0.445 ^b	77.69±0.086 ^b
KS 244+	91.67±0.362 ^a	84.28±0.317 ^b	82.99±0.355 ^b	73.30±0.167 ^b
BN 101	85.86±0.105 ^a	83.87±0.438 ^b	77.69±0.112 ^b	72.34±0.362 ^b
30V92	82.83±0.247 ^a	76.69±0.461 ^b	65.70±0.226 ^b	61.20±0.049 ^b
BN 1133	87.70±0.216 ^a	73.12±0.344 ^b	54.33±0.040 ^b	46.50±0.101 ^b
SWARNA	75.37±0.170 ^a	71.90±0.365 ^b	57.51±0.156 ^b	32.99±0.113 ^b
DHANYA	76.25±0.247 ^a	72.39±0.090 ^b	54.05±0.122 ^b	32.87±0.001 ^b

CD- Treatments: 7.97; Cultivars: 10.54 Average of three replicate experiments. $\pm$ =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 1A: ANOVA of data presented in Table 1

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	3105.635	3	1035.212	38.30806	5.06E-08	3.159908
Columns	1148.697	6	191.4496	7.084602	0.000539	2.661305
Error	486.42	18	27.02334			
Total	4740.752	27				

Table 2A: ANOVA of data presented in Table 2

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	3093.314	3	1031.105	20.48231	4.95E-06	3.159908
Columns	2861.212	6	476.8687	9.472727	9.1E-05	2.661305
Error	906.142	18	50.34122			
Total	6860.668	27				

4.2. Effect of water stress on cell membrane

4.2. Drought

Drought stress on the cell membrane injury in leaves of seven cultivars of maize *in vitro* (Fig 8) and *in vivo* (Fig 9) showed maximum cell injury during drought stress in Dhanya, Swarna and BN 1133 cultivars especially on the 7th day of stress.

4.2.2. Flooding

Cell membrane injury in the leaves of seven cultivars of maize during flooding stress showed very less electrolyte leakage in KS 244, KS 244+ and BN 101 indicating that these cultivars are more tolerant to flooding stress than the rest of the cultivars (Fig 10).

4.3. Tolerance index determination following water stress

Tolerance index from the leaves of KS 244, KS 244+ and BN 101 is maximum following water stress indicating that these cultivars among the seven cultivars of maize are most tolerant during drought stress.

Table 3: Tolerance index from the leaves of seven cultivars of maize during drought stress *in vivo*

Cultivar Treatment	DHANYA	SWARNA	BN 1133	30V92	BN 101	KS 244+	KS 244
3-DAY	0.82 ±0.0012	0.81 ±0.0010	0.85 ±0.0012	0.85 ±0.010	0.87 ±0.0003	0.86 ±0.001	0.84 ±0.001
5-DAY	0.64 ±0.0010	0.62 ±0.0010	0.65 ±0.0003	0.69 ±0.003	0.78 ±0.0003	0.77 ±0.001	0.78 ±0.002
7-DAY	0.47 ±0.0010	0.44 ±0.0012	0.48 ±0.0010	0.55 ±0.003	0.70 ±0.001	0.70 ±0.001	0.69 ±0.001

Average of three replicate experiments. ± =SE;

4.4. Lipid peroxidation of membranes following water stress

4.4.1. Drought

Lipid peroxidation from the leaves of seven cultivars of maize during drought stress *in vivo* (Fig 11) shows that there was maximum lipid peroxidation in BN 1133, Swarna, Dhanya and 30V92 which increased with the number of days of drought stress. But in case of KS 244, KS 244+ and BN 101 not much increase in lipid peroxidation was seen even during prolonged period of stress.

4.4.2. Flooding

An increase in lipid peroxidation during the period of stress was observed in the leaves of different test cultivars. 30V92, BN 1133, Swarna and Dhanya showed maximum increase in lipid peroxidation and on the 7th day the increase was double of that of the other cultivars. In KS 244+ and BN101 the increase was very insignificant (Fig 12).

4.5. Changes in carbohydrate accumulation in leaves and roots of maize cultivars following water stress

4.5.1. Drought

4.5.1.1. Total soluble sugar content in leaf and root

Total soluble sugar content in the seven cultivars during drought stress *in vitro* (Table 4) and *in vivo* (Table 5) showed an increase in both the leaves and the roots. Greater increase was seen in the roots of the test plant than in the leaves. It was seen that there was much more accumulation of sugar during drought stress in KS 244, KS 244+ and BN 101 cultivars than the rest of the cultivars.

4.5.1.2. Reducing sugar content in leaf and root

Reducing sugar content showed similar results like that of total soluble sugar in case of experiments performed both *in vitro* (Table 6) and *in vivo* (Table 7) during drought stress. Where there was an increase in the reducing sugar during the days of drought stress and maximum reducing sugar content was seen in KS 244, KS 244+ and BN 101. Greater increase in the reducing sugar content was seen in the roots of the test plant than in the leaves.

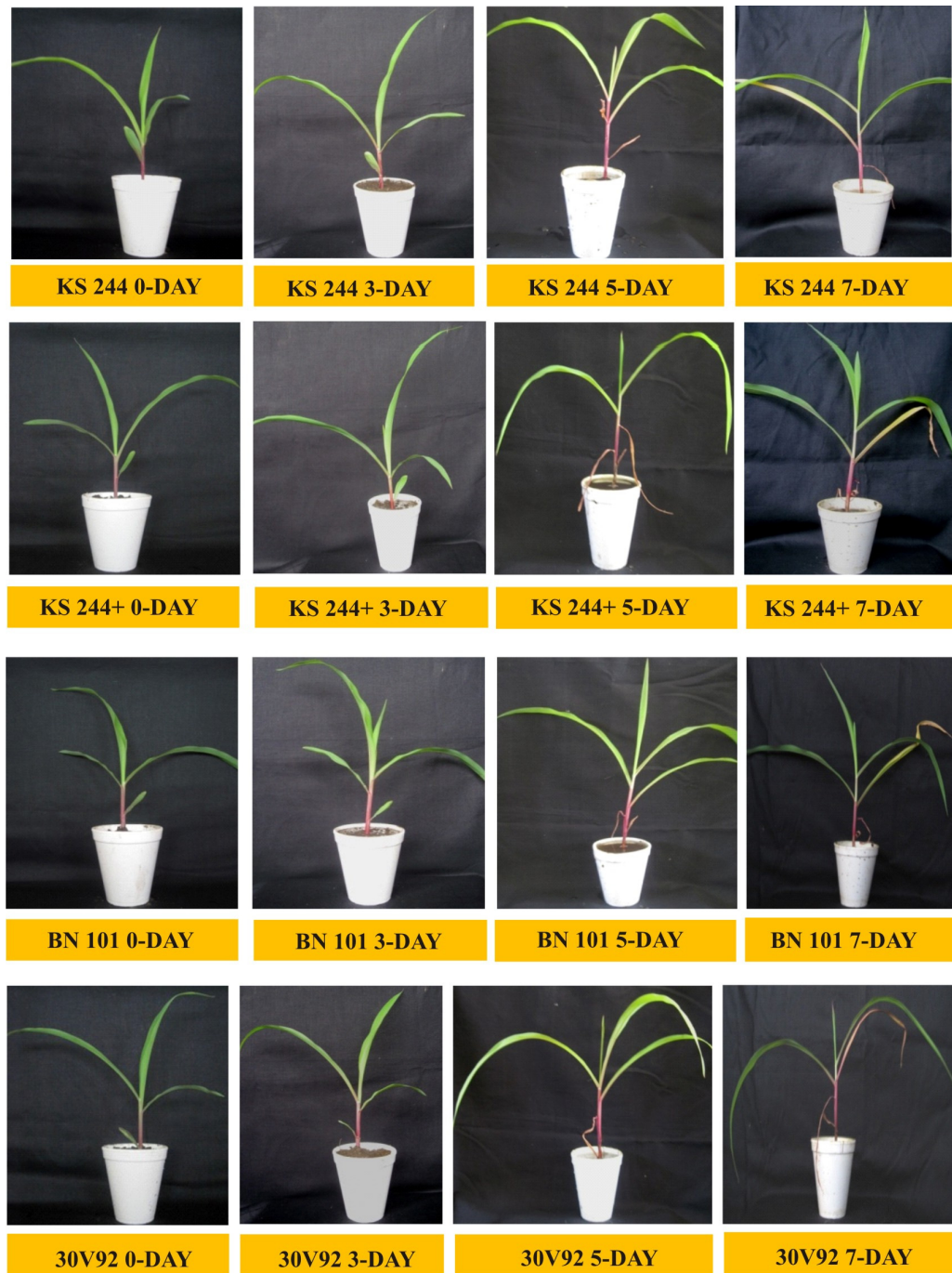


Fig. 5A: Four cultivars of maize (*Zea mays* L.) subjected to flooding stress

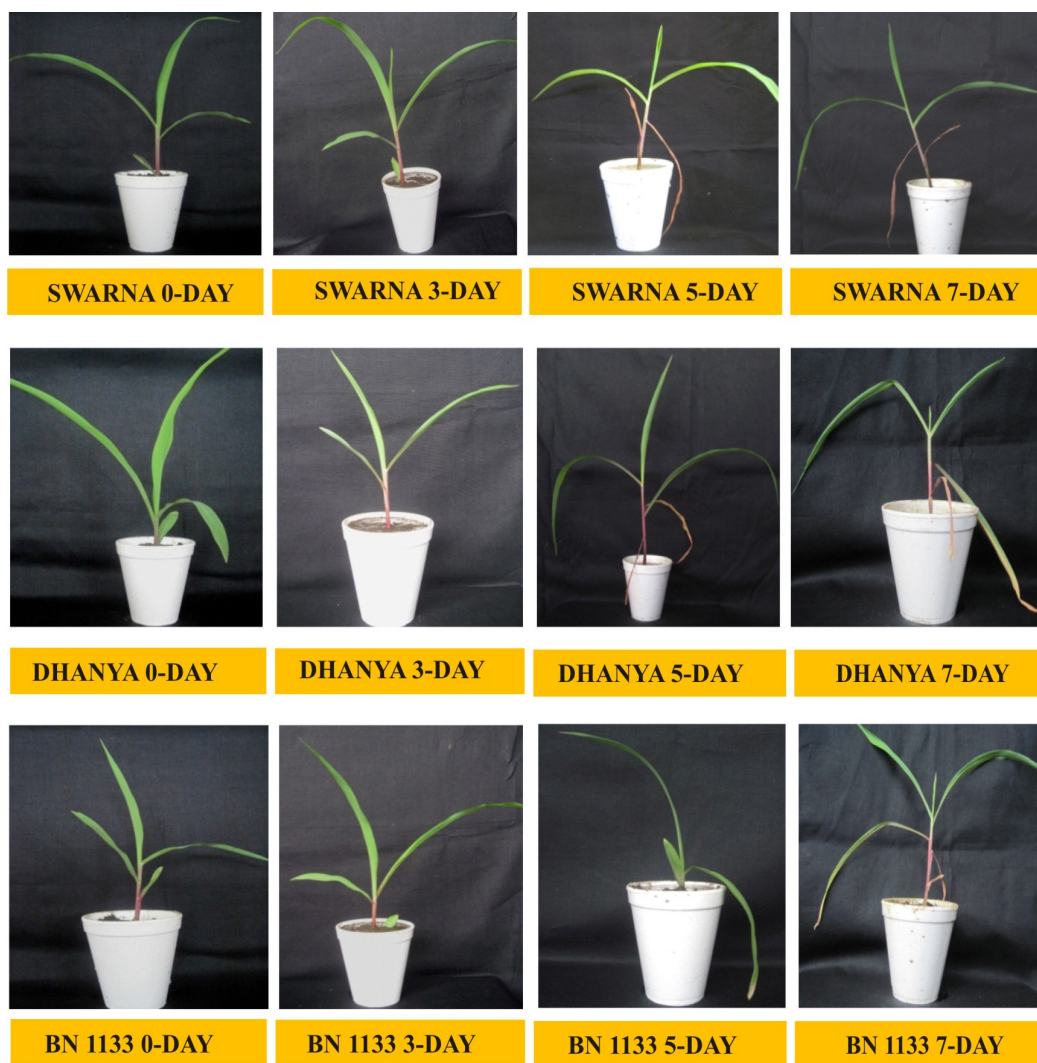


Fig. 5B: Three cultivars of maize (*Zea mays* L.) subjected to flooding stress



Fig 6A:1,2,3,4,5,6,7: Root system of KS 244, KS 244+, BN 101, 30V92, BN 1133, Swarna and Dhanya cultivars respectively on 7-day of flooding stress

Fig 6B:1,2:Root system of KS 244 and Dhanya cultivars on 7-day of flooding stress

Fig 6C: Control plants of seven cultivars of maize

Fig 6D: 5 day old plants of seven cultivars of maize during flooding stress

Fig 6E: Control leaves of Dhanya (1) leaf from plant subjected to flooding stress for 7-days (2) and 7-day leaf of Dhanya during flooding stress

Fig 6F: Control leaves of Swarna (1) leaf from plant subjected to flooding stress for 7-days (2)

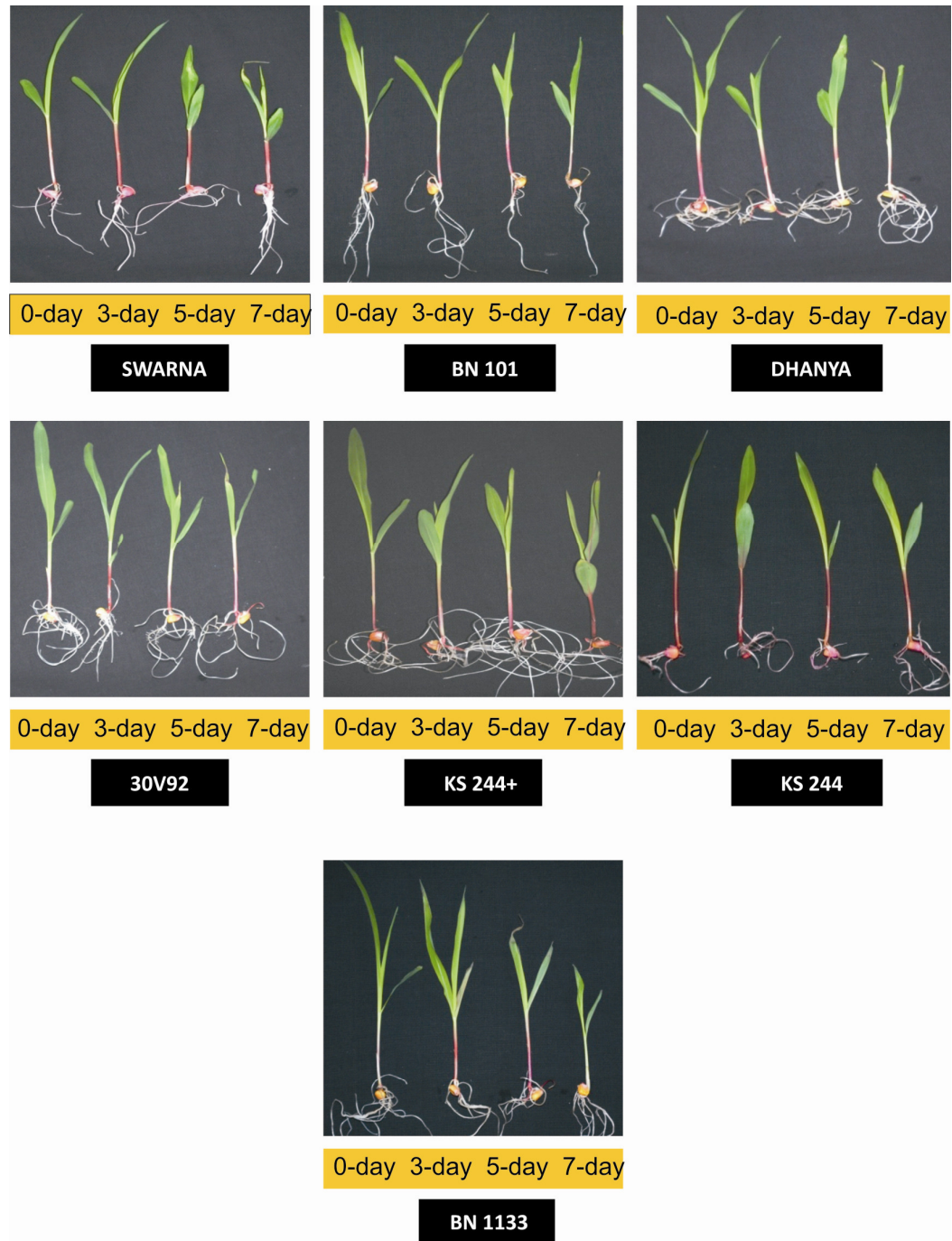
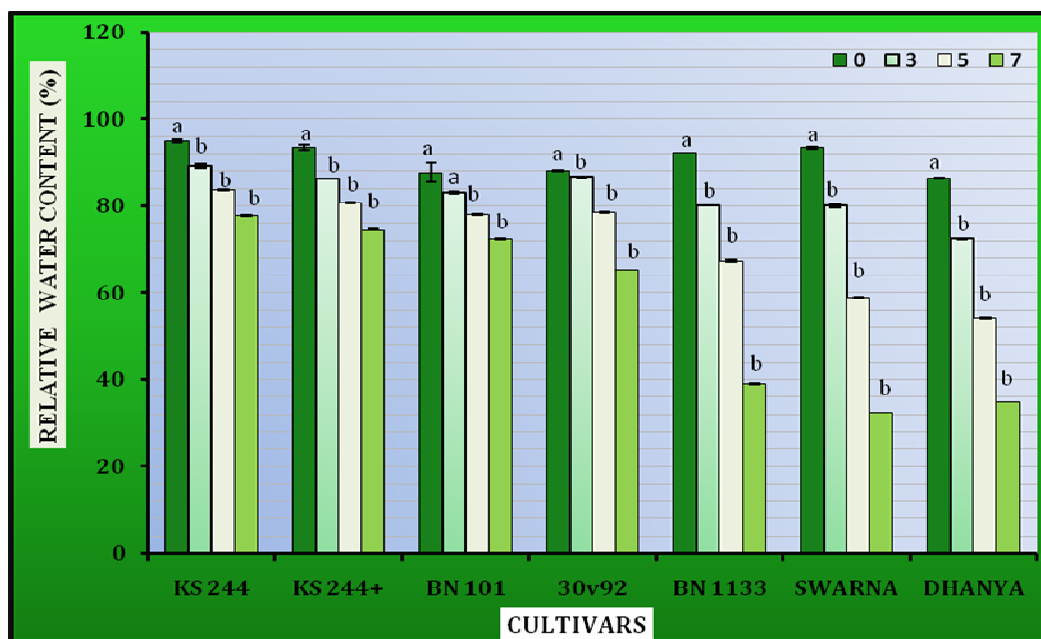


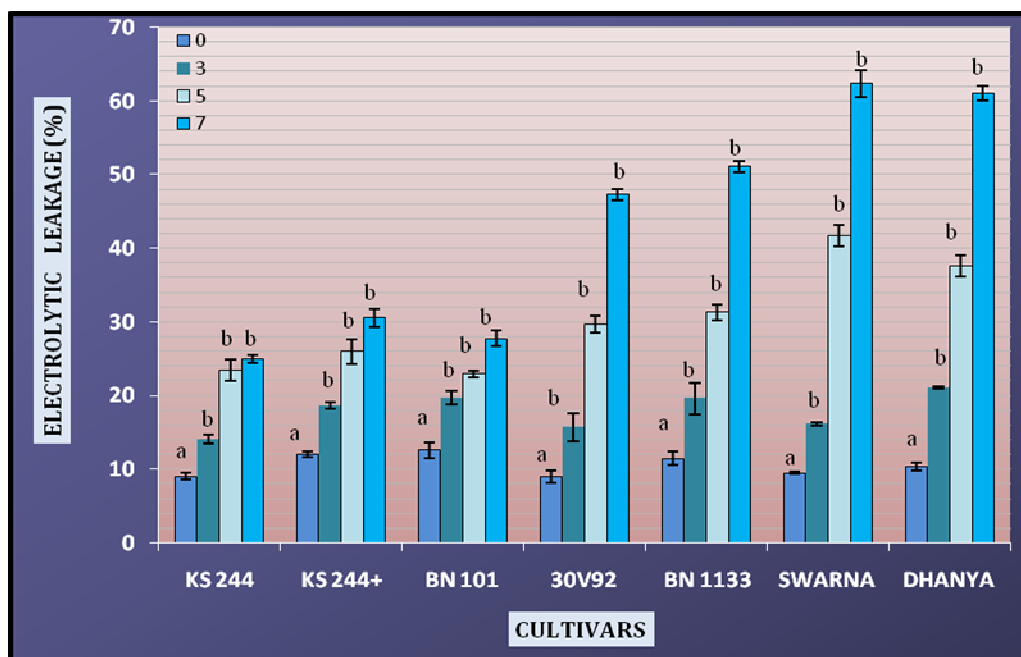
Fig.7: Seven cultivars of maize (*Zea mays* L.) subjected to drought stress *in vitro* by application of 10% PEG

Fig 4: Relative water content from the leaves of seven cultivars of maize to drought stress *in vivo*



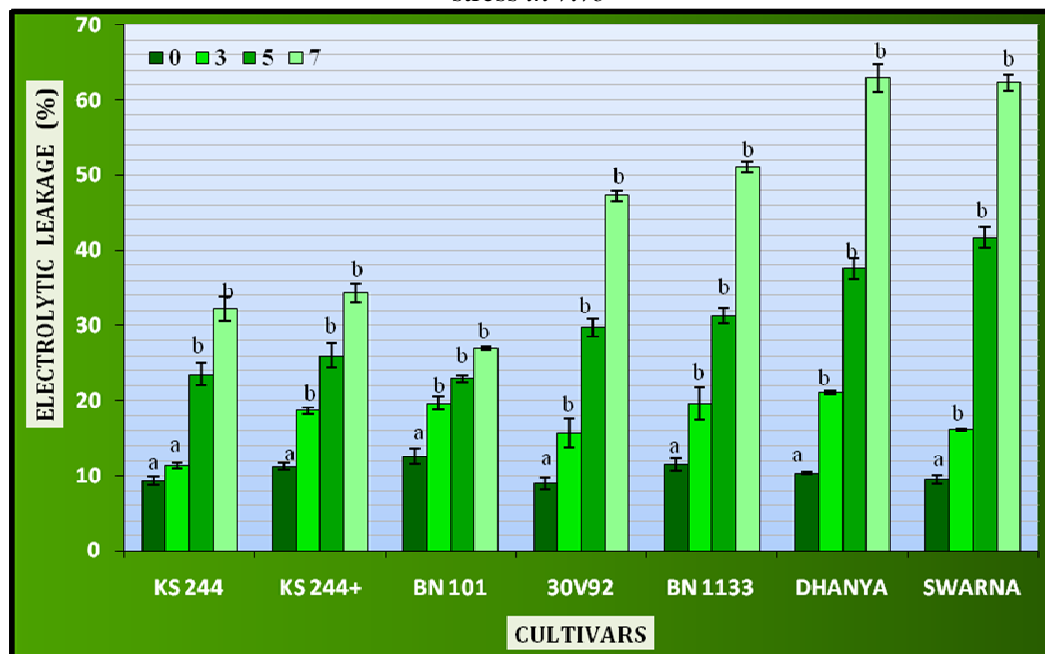
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 8: Electrolyte leakage in the leaves of seven cultivars of maize during drought stress *in vitro*



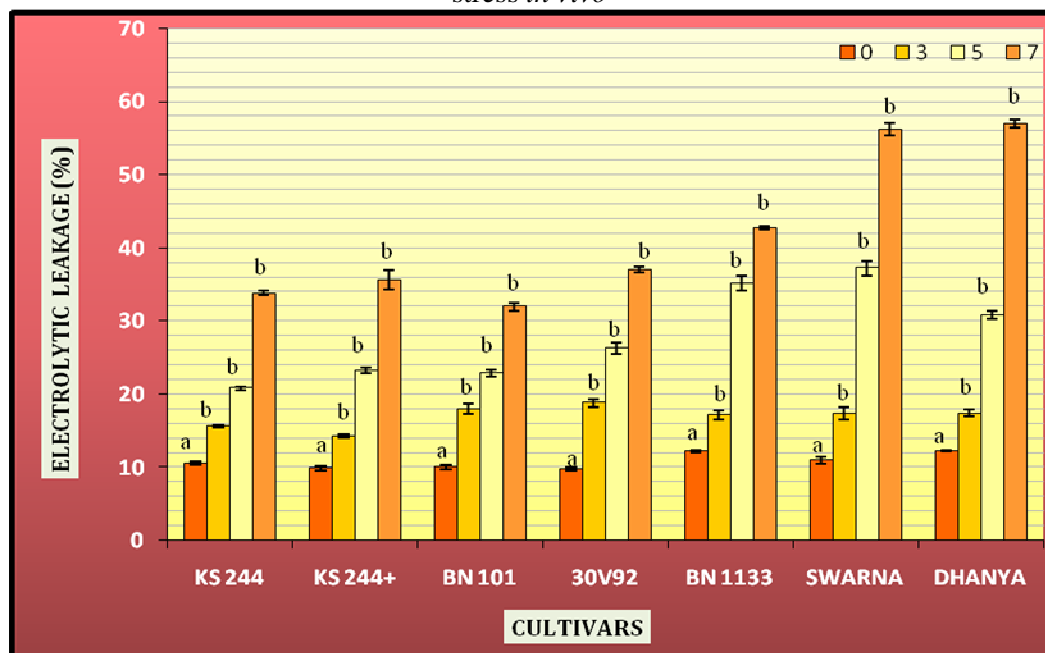
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 9: Electrolyte leakage in the leaves of seven cultivars of maize during drought stress *in vivo*

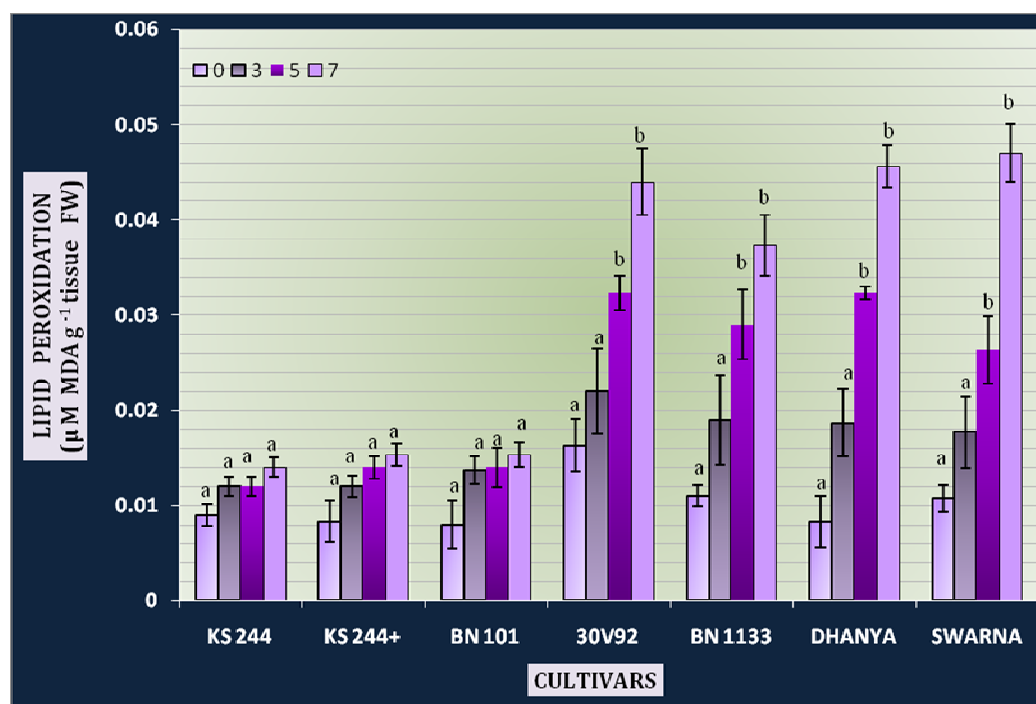


Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

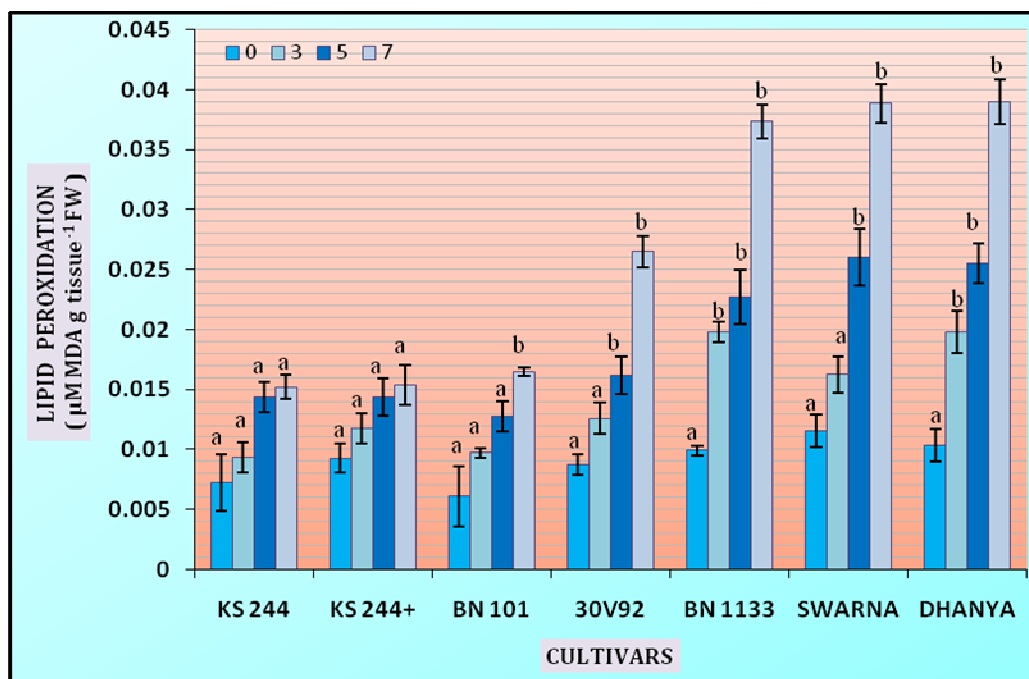
Fig 10: Electrolyte leakage in the leaves of seven cultivars of maize during flooding stress *in vivo*



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 11: Lipid peroxidation during drought stress *in vivo* in the leaves maize cultivar

Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 12: Lipid peroxidation during flooding stress from the leaves of maize cultivars

Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

4.5.1.3. Starch content in leaf and root

Starch content in the leaves and root of the seven cultivars of maize showed a decrease during drought stress both *in vitro* (Table 8) and *in vivo* (Fig 13 and Fig 14). There was a greater decrease in starch content in BN101, KS 244 and KS 244+ in both the leaves and roots of the test plant than in the other four cultivars BN 1133, Dhanya, Swarna and 30V92.

4.5.2. Flooding

4.5.2.1. Total soluble sugar content and reducing sugar in leaf and root

Total soluble sugar content (Table 9) and reducing sugar (Table 10) showed varying trend in different cultivars. In KS 244, KS 244+ and BN 101 both total sugar and reducing sugar increased with the increase in number of days of flooding stress and the increase was seen to be more in the roots of the test plant than the leaves. But in the other four cultivars ie., 30V92, Swarna, Dhanya, and BN 1133 there was an initial increase in the sugar content till the 5th day of stress but on the 7th day of stress a decrease in the sugar content was seen in both the leaves and the root of the plants, and the decrease was greater in the roots than in the shoots.

4.5.2.2. Starch content in leaf and root

Like drought stress, there was a decrease in starch content even during flooding stress in both the leaves and the root of the test cultivars. Maximum decrease was seen in BN 101, KS 244 and KS 244+, and the decrease was more in the leaves than the roots. In BN 1133, 30V92, Swarna and Dhanya very insignificant decrease was observed after the 5th day of stress in both the leaves and the root (Table 11). ANOVA of data presented in the Table 11 showed a very significant difference in the starch content among the seven cultivars.

Table 4: Total soluble sugar content in the leaves and root of maize cultivars during drought stress *in vitro*

CULTIVARS	Total soluble sugar content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	8.53± 0.009 ^a	15.33± 0.009 ^b	15.87± 0.003 ^b	18.93± 0.037 ^b	13.60± 0.010 ^a	22.53± 0.003 ^b	24.13± 0.003 ^b	24.80± 0.015 ^b
KS 244+	8.67± 0.015 ^a	9.07± 0.003 ^b	12.80± 0.006 ^b	19.47± 0.003 ^b	10.00± 0.006 ^a	14.13± 0.019 ^b	21.33± 0.007 ^b	26.80± 0.015 ^b
BN 101	8.00± 0.010 ^a	8.40± 0.006 ^b	12.93± 0.020 ^b	19.20± 0.012 ^b	11.07± 0.012 ^a	14.53± 0.012 ^b	18.00± 0.006 ^b	25.87± 0.020 ^b
30V92	6.40± 0.006 ^a	11.87± 0.012 ^b	13.6± 0.015 ^b	20.27± 0.009 ^b	9.47± 0.022 ^a	14.27± 0.012 ^b	17.20± 0.006 ^b	22.80± 0.006 ^b
BN 1133	8.93± 0.003 ^a	13.20± 0.006 ^b	14.13± 0.020 ^b	16.67± 0.003 ^b	14.93± 0.024 ^a	19.33± 0.003 ^b	21.60± 0.012 ^b	21.87± 0.003 ^b
DHANYA	5.73± 0.003 ^a	9.33± 0.003 ^b	9.87± 0.007 ^b	13.07± 0.003 ^b	8.40± 0.010 ^a	9.87± 0.003 ^b	11.33± 0.003 ^b	15.47± 0.003 ^b
SWARNA	8.13± 0.003 ^a	10.13± 0.003 ^b	11.33± 0.003 ^b	16.27± 0.007 ^b	9.73± 0.003 ^a	12.27± 0.003 ^b	14.27± 0.003 ^b	22.93± 0.012 ^b

LEAF: CD- Treatments: 1.03; Cultivars: 1.9 ROOT: CD- Treatments: 2.54; Cultivars: 3.36
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P <0.05 in Students 't' test within each cultivar.

Table 4A: ANOVA of data presented in Table 4 (Total soluble sugar of leaf)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	194.7026	3	64.90085	38.69008	4.68E-08	3.159908
Columns	143.2219	6	23.87032	14.23008	5.75E-06	2.661305
Error	30.19418	18	1.677455			
Total	368.1186	27				

Table 4B: ANOVA of data presented in Table 4 (Total soluble sugar of root)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	527.6394	3	175.8798	34.4593	1.14E-07	3.159908
Columns	256.5575	6	42.75958	8.377683	0.000197	2.661305
Error	91.87175	18	5.103986			
Total	876.0686	27				

Table 5: Total soluble sugar during drought stress in leaves and root of seven cultivars of maize *in vivo*

CULTIVARS	Total soluble sugar content (mg g⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	2.80± 0.231 ^a	5.30± 0.353 ^b	10.60± 0.481 ^b	12.67± 0.133 ^b	3.47± 0.133 ^a	7.06± 0.133 ^b	12.8± 0.611 ^b	15.20± 0.692 ^b
KS 244+	2.13± 0.874 ^a	4.13± 0.133 ^a	6.27± 0.133 ^b	11.87± 0.267 ^b	3.87± 0.133 ^a	6.27± 0.133 ^b	13.33± 0.353 ^b	15.73± 0.133 ^b
BN 101	4.4± 0.231 ^a	4.53± 1.272 ^a	7.86± 0.267 ^b	14.0± 0.400 ^b	5.60± 0.231 ^a	6.67± 0.481 ^a	8.67± 0.267 ^b	14.93± 0.133 ^b
30V92	2.53± 0.667 ^a	2.40± 1.001 ^a	5.47± 0.133 ^b	8.60± 0.133 ^b	3.20± 0.400 ^a	4.93± 0.133 ^b	10.67± 0.353 ^b	12.67± 0.133 ^b
BN 1133	2.53± 0.353 ^a	3.33± 0.267 ^a	6.27± 0.481 ^b	9.73± 0.133 ^b	3.73± 0.133 ^a	7.33± 0.133 ^b	8.67± 0.133 ^b	11.6± 0.400 ^b
DHANYA	3.06± 0.267 ^a	4.00± 0.231 ^a	5.06± 0.267 ^b	7.73± 0.353 ^b	3.60± 0.231 ^a	5.30± 0.133 ^b	6.90± 0.353 ^b	8.27± 0.133 ^b
SWARNA	1.47± 0.133 ^a	2.27± 0.133 ^b	4.27± 0.267 ^b	5.07± 0.133 ^b	1.73± 0.133 ^a	3.07± 0.133 ^b	3.87± 0.133 ^b	8.13± 0.742 ^b

LEAF: CD- Treatments: 1.52; Cultivars: 2.01 ROOT: CD- Treatments: 1.86; Cultivars: 2.47

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 5A: ANOVA of data presented in Table 5 (Total soluble sugar of leaf)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	221.4775	3	73.82582	40.31439	3.4E-08	3.159908
Columns	65.18603	6	10.86434	5.932737	0.001458	2.661305
Error	32.96254	18	1.831252			
Total	319.626	27				

Table 6: Reducing sugar in the leaf and root of seven cultivars of maize during drought stress *in vitro*

CULTIVARS	Reducing sugar content (mg g⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	6.67± 0.011 ^a	11.04± 0.022 ^b	14.07± 0.024 ^b	15.93± 0.017 ^b	11.78± 0.017 ^a	14.00± 0.012 ^b	17.04± 0.033 ^b	17.56± 0.010 ^b
KS 244+	3.19± 0.009 ^a	7.33± 0.006 ^b	10.15± 0.018 ^b	12.15± 0.003 ^b	5.26± 0.003 ^a	9.41± 0.015 ^b	12.00± 0.015 ^b	12.74± 0.009 ^b
BN 101	2.44± 0.006 ^a	8.00± 0.006 ^b	9.56± 0.021 ^b	12.22± 0.006 ^b	4.22± 0.012 ^a	9.33± 0.006 ^b	11.56± 0.012 ^b	14.81± 0.018 ^b
30V92	3.11± 0.006 ^a	4.15± 0.003 ^b	5.48± 0.009 ^b	10.67± 0.006 ^b	4.67± 0.006 ^a	5.04± 0.009 ^b	6.96± 0.009 ^b	11.33± 0.006 ^b
BN 1133	4.00± 0.006 ^a	4.89± 0.006 ^b	7.26± 0.012 ^b	10.07± 0.009 ^b	5.78± 0.006 ^a	8.15± 0.003 ^b	8.96± 0.003 ^b	10.52± 0.009 ^b
DHANYA	2.44± 0.006 ^a	3.56± 0.006 ^b	4.96± 0.009 ^b	6.07± 0.007 ^b	4.00± 0.006 ^a	5.04± 0.009 ^b	5.70± 0.003 ^b	6.96± 0.003 ^b
SWARNA	2.89± 0.006 ^a	6.81± 0.007 ^b	7.11± 0.006 ^b	8.15± 0.009 ^b	4.15± 0.003 ^a	8.07± 0.009 ^b	9.04± 0.007 ^b	9.93± 0.009 ^b

LEAF: CD- Treatments: 1.45; Cultivars: 1.92 ROOT: CD- Treatments: 1.42; Cultivars: 1.89
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 5B: ANOVA of data presented in Table 5 (Total soluble sugar of root)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	312.1041	3	104.0347	37.76613	5.64E-08	3.159908
Columns	99.19492	6	16.53249	6.001537	0.00137	2.661305
Error	49.58476	18	2.754709			
Total	460.8838	27				

Table 6A: ANOVA of data presented in Table 6 (Reducing sugar in leaf)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	194.7026	3	64.90085	38.69008	4.68E-08	3.159908
Columns	143.2219	6	23.87032	14.23008	5.75E-06	2.661305
Error	30.19418	18	1.677455			
Total	368.1186	27				

Table 6B: ANOVA of data presented in Table 6 (Reducing sugar in root)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	150.5051	3	50.16837	31.03747	2.5E-07	3.159908
Columns	229.6591	6	38.27652	23.68039	1.28E-07	2.661305
Error	29.09485	18	1.616381			
Total	409.2591	27				

Table 7A: ANOVA of data presented in Table 7 (Reducing sugar in leaf)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	33.92035	3	11.30678	46.88489	1.04E-08	3.159908
Columns	15.13119	6	2.521865	10.4572	4.78E-05	2.661305
Error	4.340889	18	0.241161			
Total	53.39243	27				

Table 7: Reducing sugar in the leaf and root of seven cultivars of maize during drought stress *in vivo*

CULTIVARS	Reducing sugar content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	0.30± 0.074 ^a	1.93± 0.074 ^b	2.30± 0.074 ^b	3.25± 0.074 ^b	1.04± 0.074 ^a	2.89± 0.13 ^b	3.85± 0.074 ^b	6.07± 0.074 ^b
KS 244+	0.67± 0.257 ^a	1.54± 0.128 ^a	2.96± 0.148 ^b	5.19± 0.322 ^b	1.19± 0.296 ^a	2.15± 0.148 ^a	5.56± 0.128 ^b	6.52± 0.196 ^b
BN 101	1.48± 0.148 ^a	2.89± 0.128 ^b	4.07± 0.074 ^b	5.63± 0.074 ^b	2.15± 0.074 ^a	2.67± 0.128 ^b	4.30± 0.195 ^b	6.20± 0.128 ^b
30V92	0.59± 0.074 ^a	1.26± 0.323 ^a	2.30± 0.196 ^b	3.63± 0.074 ^b	1.11± 0.222 ^a	2.15± 0.148 ^b	3.93± 0.074 ^b	5.04± 0.267 ^b
BN 1133	0.59± 0.148 ^a	1.11± 0.257 ^a	1.85± 0.074 ^b	3.11± 0.128 ^b	1.26± 0.412 ^a	2.81± 0.322 ^a	3.56± 0.128 ^b	4.81± 0.267 ^b
DHANYA	0.51± 0.074 ^a	1.04± 0.074 ^b	1.26± 0.074 ^b	2.30± 0.074 ^b	0.59± 0.074 ^a	1.40± 0.07 ^b	1.70± 0.074 ^b	2.81± 0.196 ^b
SWARNA	0.29± 0.074 ^a	0.96± 0.074 ^b	1.63± 0.148 ^b	2.30± 0.074 ^b	0.51± 0.074 ^a	1.26± 0.074 ^b	2.29± 0.148 ^b	2.51± 0.074 ^b

LEAF: CD- Treatments: 0.55; Cultivars: 0.73 ROOT: CD- Treatments: 0.77; Cultivars: 1.02
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 7B: ANOVA of data presented in Table 7 (Reducing sugar in root)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	55.83271	3	18.6109	39.20558	4.23E-08	3.159908
Columns	21.32585	6	3.554309	7.487478	0.000389	2.661305
Error	8.544607	18	0.4747			
Total	85.70317	27				

Table 8: Starch content in seven cultivars of maize during drought stress *in vitro*

CULTIVARS	Starch content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	29.70± 0.4903 ^a	19.52± 0.490 ^b	15.84± 0.283 ^b	10.74± 0.283 ^b	29.13± 0.283 ^a	18.38± 0.283 ^b	16.40± 0.283 ^b	7.35± 1.414 ^b
KS 244+	27.43± 0.748 ^a	18.95± 0.748 ^b	10.18± 0.490 ^b	8.77± 0.566 ^b	28.00± 0.980 ^a	18.38± 0.283 ^b	14.14± 0.748 ^b	10.46± 0.283 ^b
BN 101	26.59± 0.282 ^a	14.42± 0.490 ^b	12.44± 0.283 ^b	9.62± 0.283 ^b	31.11± 0.283 ^a	23.19± 0.748 ^b	16.68± 0.566 ^b	11.03± 0.490 ^b
30V92	33.66± 1.575 ^a	25.74± 0.283 ^b	16.40± 0.748 ^b	9.05± 0.566 ^b	39.60± 0.283 ^a	29.98± 0.566 ^b	20.65± 1.575 ^b	12.16± 0.566 ^b
BN 1133	32.24± 1.296 ^a	26.87± 0.748 ^b	20.36± 0.849 ^b	10.18± 0.490 ^b	39.88± 0.490 ^a	31.39± 0.980 ^b	26.59± 0.748 ^b	17.82± 0.490 ^b
DHANYA	35.92± 0.748 ^a	31.11± 0.748 ^b	21.49± 0.283 ^b	12.73± 0.849 ^b	41.86± 1.131 ^a	34.79± 0.980 ^b	25.74± 0.748 ^b	17.25± 1.020 ^b
SWARNA	31.39± 0.490 ^a	26.87± 0.282 ^b	21.21± 1.296 ^b	15.27± 0.490 ^b	38.75± 1.131 ^a	33.37± 0.748 ^b	26.59± 1.020 ^b	16.97± 0.849 ^b

LEAF: CD- Treatments: 2.33; Cultivars: 3.08 ROOT: CD- Treatments: 3.40; Cultivars: 4.50
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 8A: ANOVA of data presented in Table 8 (Starch content in leaf)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	1917.561	3	639.187	148.5733	6.93E-13	3.159908
Columns	460.6467	6	76.77445	17.84553	1.1E-06	2.661305
Error	77.43898	18	4.302166			
Total	2455.647	27				

Table 9: Total soluble sugar content in the leaves and root of maize cultivars during flooding stress

CULTIVARS	Total soluble sugar content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	7.33± 0.27 ^a	8.93± 0.133 ^b	14.13± 0.353 ^b	18.67± 0.933 ^b	8.93± 0.353 ^a	11.73± 0.133 ^b	15.87± 0.933 ^b	21.20± 0.400 ^b
KS 244+	8.27± 0.353 ^a	10.00± 0.231 ^b	13.87± 0.133 ^b	18.27± 0.267 ^b	10.53± 0.133 ^a	11.60± 0.231 ^b	15.87± 0.133 ^b	20.80± 0.400 ^b
BN 101	6.40± 0.231 ^a	10.80± 0.400 ^b	13.33± 0.481 ^b	18.00± 0.231 ^b	9.60± 0.231 ^a	12.80± 0.231 ^b	15.87± 1.272 ^b	24.00± 0.833 ^b
30V92	5.73± 0.133 ^a	7.47± 0.133 ^b	10.53± 0.353 ^b	8.27± 0.581 ^b	5.2± 0.231 ^a	10.53± 0.667 ^b	13.07± 0.267 ^b	5.60± 0.231 ^a
BN 1133	6.27± 0.353 ^a	11.07± 0.353 ^b	11.47± 0.133 ^b	7.07± 0.353 ^a	9.47± 0.267 ^a	11.47± 0.481 ^b	14.67± 0.351 ^b	5.20± 0.231 ^b
DHANYA	4.93± 0.133 ^a	6.4± 0.231 ^b	10.4± 0.231 ^b	8.8± 0.231 ^b	7.07± 0.353 ^a	8.93± 1.139 ^a	10.93± 0.353 ^b	4.80± 0.230 ^b
SWARNA	3.73± 0.13 ^a	6.67± 0.35 ^a	11.47± 0.27 ^a	9.20± 0.46 ^a	6.53± 0.267 ^a	10.27± 0.481 ^b	11.47± 0.481 ^b	5.20± 0.400 ^a

LEAF: CD- Treatments: 1.31; Cultivars: 1.74 ROOT: CD- Treatments: 1.30; Cultivars: 1.72
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 8B: ANOVA of data presented in Table 8 (Starch content in root)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	1954.754	3	651.5846	71.02142	3.59E-10	3.159908
Columns	942.8098	6	157.135	17.1274	1.5E-06	2.661305
Error	165.1406	18	9.17448			
Total	3062.704	27				

Table 10: Reducing sugar content in the leaves and root of maize cultivars in flooding

CULTIVARS	Reducing sugar content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	2.44± 0.128 ^a	4.15± 0.074 ^b	5.63± 0.519 ^b	7.19± 0.074 ^b	3.26± 0.074 ^a	4.96± 0.074 ^b	6.52± 0.633 ^b	9.41± 0.074 ^b
KS 244+	2.37± 0.148 ^a	3.63± 0.074 ^b	5.33± 0.128 ^b	6.96± 0.074 ^b	3.04± 0.074 ^a	4.59± 0.074 ^b	6.15± 0.196 ^b	8.22± 0.667 ^b
BN 101	2.15± 0.148 ^a	2.89± 0.128 ^b	5.11± 0.222 ^b	7.11± 0.222 ^b	2.44± 0.222 ^a	3.48± 0.074 ^b	6.30± 0.074 ^b	8.00± 0.1283 ^b
30V92	1.332± 0.128 ^a	2.81± 0.148 ^b	3.41± 0.196 ^b	3.33± 0.128 ^b	2.15± 0.074 ^a	3.48± 0.196 ^b	4.74± 0.074 ^b	2.52± 0.1481 ^a
BN 1133	2.00± 0.128 ^a	2.81± 0.148 ^b	4.52± 0.296 ^b	3.56± 0.222 ^b	2.30± 0.148 ^a	4.96± 0.196 ^b	6.07± 0.148 ^b	2.81± 0.074 ^a
DHANYA	1.78± 0.128 ^a	3.04± 0.074 ^b	4.963± 0.074 ^b	3.56± 0.128 ^b	2.30± 0.196 ^a	5.26± 0.392 ^b	6.22± 0.128 ^b	2.81± 0.0741 ^a
SWARNA	1.63± 0.074 ^a	3.56± 0.128 ^b	4.74± 0.074 ^b	3.19± 0.074 ^b	2.15± 0.148 ^a	4.44± 0.222 ^b	4.89± 0.128 ^b	2.07± 0.074 ^a

LEAF: CD- Treatments: 0.96; Cultivars: 1.27 ROOT: CD- Treatments: 1.73; Cultivars: 2.29
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 9A: ANOVA of data presented in Table 9 (Starch content in leaf)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	204.4616	3	68.15386	49.94342	6.29E-09	3.159908
Columns	8.860263	6	1.47671	1.082139	0.409466	2.661305
Error	24.56319	18	1.364621			
Total	237.885	27				

Table 11: Starch content in the leaves and root of maize cultivars in flooding stress

CULTIVARS	Starch content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	15.21± 0.464 ^a	11.74± 0.141 ^b	8.35± 0.071 ^b	5.59± 0.071 ^b	18.18± 0.071 ^a	13.73± 0.255 ^b	8.77± 0.071 ^b	6.37± 0.212 ^b
KS 244+	15.84± 0.255 ^a	13.86± 0.071 ^b	9.12± 0.212 ^b	6.36± 0.123 ^b	18.32± 0.566 ^a	14.78± 0.283 ^b	11.32± 0.255 ^b	8.42± 0.187 ^b
BN 101	14.85± 0.212 ^a	11.46 ±0.424 ^b	7.92± 0.141 ^b	5.38± 0.071 ^b	18.18± 0.187 ^a	13.93± 0.141 ^b	11.46± 0.245 ^b	8.28± 0.212 ^b
30V92	13.09± 0.071 ^a	11.32± 0.374 ^b	9.62± 0.464 ^b	6.86± 0.187 ^b	16.13± 0.534 ^a	14.15± 0.308 ^b	11.88± 0.245 ^b	10.19± 0.368 ^b
BN 1133	14.36± 0.071 ^a	10.96± 0.071 ^b	9.62± 0.141 ^b	9.12± 0.122 ^b	18.39± 0.071 ^a	15.21± 0.071 ^b	14.50± 0.071 ^b	12.87± 0.308 ^b
DHANYA	14.00± 0.212 ^a	11.39± 0.604 ^b	10.19± 0.123 ^b	9.47± 0.255 ^b	19.45± 0.374 ^a	15.99± 0.374 ^b	14.22± 0.245 ^b	12.94± 0.245 ^b
SWARNA	14.85± 0.122 ^a	11.39± 0.308 ^b	9.83± 0.187 ^b	9.12± 0.122 ^b	19.03± 0.071 ^a	15.56± 0.495 ^b	13.44± 0.354 ^b	12.10± 0.212 ^b

LEAF: CD- Treatments: 1.31; Cultivars: 1.74 ROOT: CD- Treatments: 1.30; Cultivars: 1.72
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 9B: ANOVA of data presented in Table 9 (Starch content in root)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	254.1635	3	84.72117	63.33054	9.21E-10	3.159908
Columns	50.91118	6	8.485196	6.342832	0.001011	2.661305
Error	24.07971	18	1.337762			
Total	329.1544	27				

Table 10A: ANOVA of data presented in Table 10 (Reducing sugar of leaf)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	42.69717	3	14.23239	19.34719	7.29E-06	3.159908
Columns	15.77315	6	2.628859	3.57361	0.01648	2.661305
Error	13.24136	18	0.735631			
Total	71.71168	27				

Table 10B: ANOVA of data presented in Table 10 (Reducing sugar of root)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	42.78868	3	14.26289	5.995031	0.005118	3.159908
Columns	27.49857	6	4.583095	1.926383	0.131295	2.661305
Error	42.82415	18	2.379119			
Total	113.1114	27				

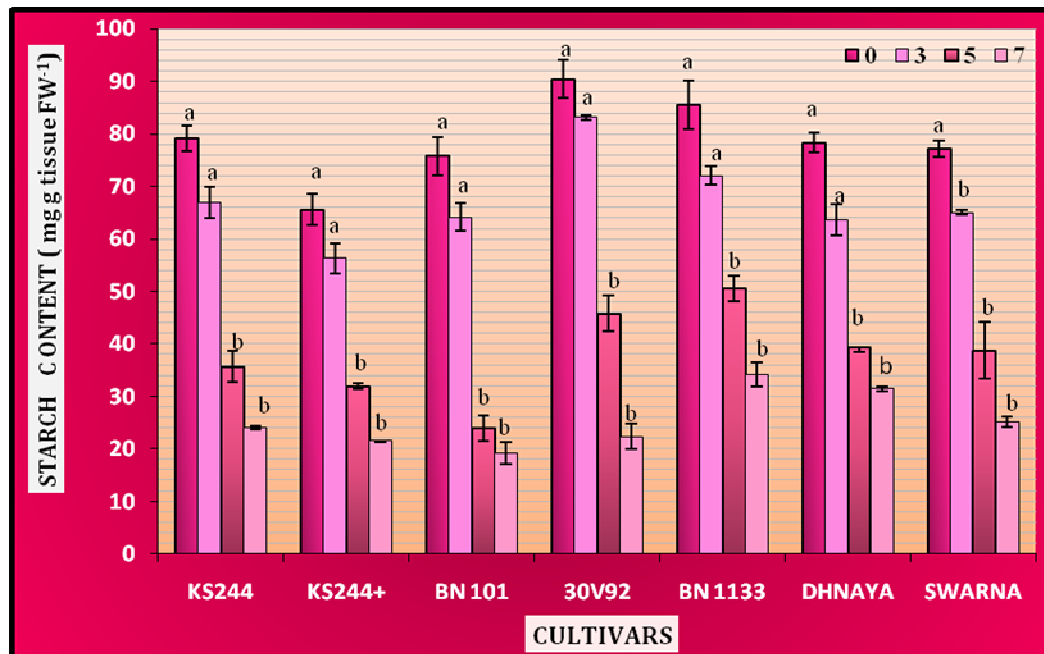
Table 11A: ANOVA of data presented in Table 11 (Starch content of leaves)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	204.4616	3	68.15386	49.94342	6.29E-09	3.159908
Columns	8.860263	6	1.47671	1.082139	0.409466	2.661305
Error	24.56319	18	1.364621			
Total	237.885	27				

Table 11B: ANOVA of data presented in Table 11 (Starch content of root)

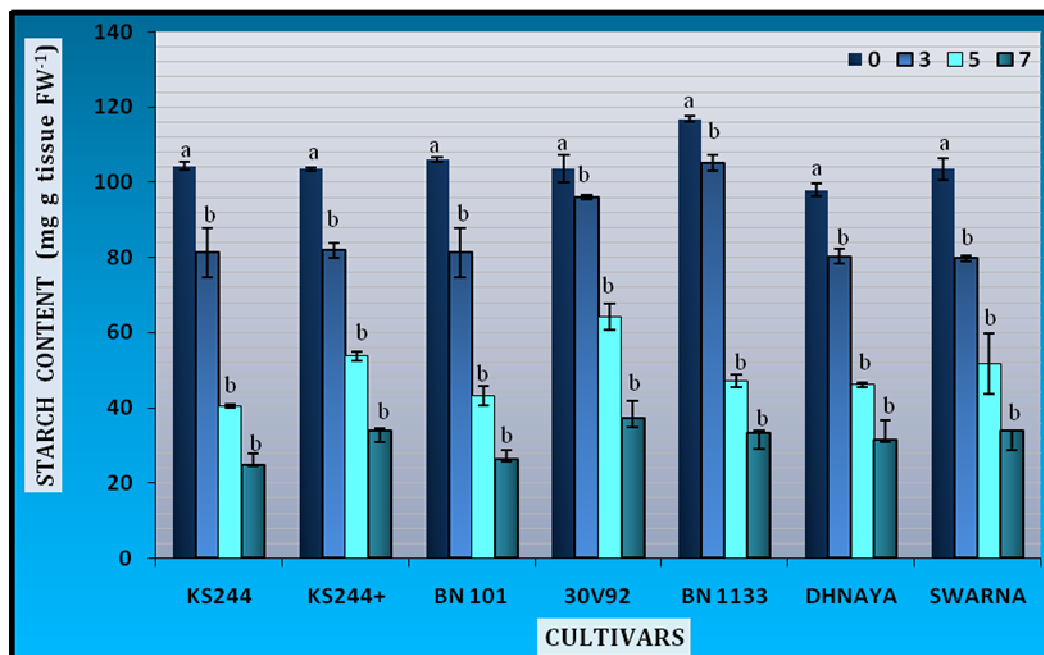
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	254.1635	3	84.72117	63.33054	9.21E-10	3.159908
Columns	50.91118	6	8.485196	6.342832	0.001011	2.661305
Error	24.07971	18	1.337762			
Total	329.1544	27				

Fig 13: Starch content in leaves of maize cultivars during drought stress *in vivo*



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 14: Starch content in the root of maize cultivars during drought stress *in vivo*



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

4.6. Effect of water stress in proline accumulation in leaves and roots of maize cultivars

Proline content in the leaves and root of seven cultivars of maize plant showed an increase during drought stress both *in vitro* (Table 12) and *in vivo* (Table 13) conditions. Greater increase in proline content was seen in the leaves of the test plant than the roots. Among all the cultivars maximum proline content was seen in KS 244, KS 244+ and BN 101 during drought stress. In BN 1133, Swarna, Dhanya and 30V92 cultivars the increase in proline accumulation was almost half of the other cultivars during prolonged drought. ANOVA of data presented in Table 12 and Table 13 showed a significant difference among the seven cultivars of maize. In flooding stress there was a decrease in proline content on the 7th day of flooding in both the leaves and the root of 30V92, BN 1133, Swarna and Dhanya cultivars (Table 14).

4.7. Influence of water stress on chlorophyll contents following water stress

4.7.1. Drought

Drought stress led to a decrease in the total chlorophyll, chlorophyll-a and chlorophyll-b content in the leaves of seven cultivars of test plant both *in vitro* (Table 15) and *in vivo* (Table 16) conditions. Maximum decrease was observed in case of Dhanya, Swarna and BN 1133, the decrease in chlorophyll content in these cultivars was twice of that of the other cultivars that is- KS 44, KS 244+, BN 101 and 30V92.

4.7.2. Flooding

Like drought stress, flooding stress also led to a significant decrease in the chlorophyll content of in the leaves of seven cultivars of test plant. Maximum decrease during flooding stress was observed in Dhanya, Swarna and BN 1133 (Table 17).

Table 12: Proline content in the leaves and root of seven maize cultivars during drought stress *in vitro*

CULTIVARS	Proline content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	0.75± 0.011 ^a	1.25± 0.023 ^b	1.6± 0.035 ^b	2.48± 0.017 ^b	0.35± 0.006 ^a	1.40± 0.006 ^b	1.58± 0.054 ^b	2.03± 0.050 ^b
KS 244+	0.59± 0.013 ^a	1.16± 0.019 ^b	1.45± 0.023 ^b	2.35± 0.035 ^b	0.42± 0.051 ^a	1.37± 0.022 ^b	1.62± 0.015 ^b	1.88± 0.029 ^b
BN 101	0.88± 0.011 ^a	1.50± 0.011 ^b	1.88± 0.006 ^b	2.63± 0.006 ^b	0.75± 0.011 ^a	1.25± 0.017 ^b	1.63± 0.011 ^b	2.50± 0.033 ^b
30V92	0.63± 0.017 ^a	0.75± 0.011 ^b	0.95± 0.031 ^b	0.98± 0.017 ^b	0.22± 0.017 ^a	0.68± 0.027 ^b	1.16± 0.006 ^b	1.52± 0.076 ^b
BN 1133	0.18± 0.006 ^a	0.19± 0.013 ^a	0.26± 0.011 ^b	0.46± 0.006 ^b	0.11± 0.019 ^a	0.13± 0.027 ^a	0.28± 0.017 ^b	0.59± 0.025 ^b
DHANYA	0.09± 0.006 ^a	0.28± 0.025 ^b	0.38± 0.035 ^b	0.84± 0.025 ^b	0.44± 0.025 ^a	0.28± 0.011 ^b	0.56± 0.011 ^b	0.84± 0.011 ^b
SWARNA	0.23± 0.011 ^a	0.25± 0.006 ^a	0.30± 0.004 ^b	0.70± 0.017 ^b	0.23± 0.017 ^a	0.25± 0.017 ^a	0.34± 0.017 ^b	0.60± 0.011 ^b

LEAF: CD- Treatments: 0.33; Cultivars: 0.44 ROOT: CD- Treatments: 0.33; Cultivars: 0.44
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 12A: ANOVA of data presented in Table 12 (Proline content in leaf)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	3.844129	3	1.281376	14.68068	4.41E-05	3.159908
Columns	8.923453	6	1.487242	17.03928	1.56E-06	2.661305
Error	1.571097	18	0.087283			
Total	14.33868	27				

Table 13: Proline content in the leaves and root of maize cultivars during drought stress *in vivo*

CULTIVARS	Proline content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	0.41± 0.032 ^a	0.50± 0.063 ^a	0.94± 0.108 ^b	1.56± 0.063 ^b	0.12± 0.006 ^a	0.16± 0.006 ^a	0.32± 0.029 ^b	0.41± 0.022 ^b
KS 244+	0.45± 0.015 ^a	0.50± 0.025 ^a	0.81± 0.013 ^b	1.55± 0.070 ^b	0.12± 0.050 ^a	0.18± 0.013 ^a	0.31± 0.017 ^b	0.48± 0.006 ^b
BN 101	0.39± 0.011 ^a	0.47± 0.025 ^a	0.90± 0.008 ^b	1.19± 0.004 ^b	0.11± 0.010 ^a	0.26± 0.038 ^b	0.36± 0.011 ^b	0.41± 0.006 ^b
30V92	0.26± 0.025 ^a	0.30± 0.009 ^a	0.46± 0.019 ^b	0.73± 0.028 ^b	0.075± 0.011 ^a	0.10± 0.006 ^a	0.26± 0.011 ^a	0.34± 0.006 ^b
BN 1133	0.40± 0.017 ^a	0.44± 0.025 ^a	0.48± 0.017 ^b	0.53± 0.011 ^b	0.006± 0.001 ^a	0.12± 0.055 ^b	0.19± 0.005 ^a	0.26± 0.014 ^a
DHANYA	0.28± 0.018 ^a	0.35± 0.005 ^b	0.48± 0.005 ^a	0.60± 0.005 ^a	0.08± 0.006 ^a	0.11± 0.011 ^a	0.17± 0.011 ^b	0.19± 0.013 ^b
SWARNA	0.38± 0.049 ^a	0.46± 0.013 ^a	0.54± 0.006 ^b	0.51± 0.031 ^a	0.11± 0.011 ^a	0.23± 0.011 ^b	0.27± 0.006 ⁶	0.28± 0.011 ^b

LEAF: CD- Treatments: 0.23; Cultivars: 0.31 ROOT: CD- Treatments: 0.054; Cultivars: 0.071
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 12B: ANOVA of data presented in Table 12 (Proline content in root)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	4.184245	3	1.394748	16.2138	2.35E-05	3.159908
Columns	6.355036	6	1.059173	12.31277	1.59E-05	2.661305
Error	1.548401	18	0.086022			
Total	12.08768	27				

Table 13A: ANOVA of data presented in Table 13 (Proline content in leaf)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	1.468588	3	0.489529	11.40705	0.000201	3.159908
Columns	0.906751	6	0.151125	3.521528	0.017509	2.661305
Error	0.772463	18	0.042915			
Total	3.147803	27				

Table 14: Proline content in the leaves and root of maize cultivars during flooding

CULTIVARS	Proline content (mg g⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	0.16± 0.017 ^a	0.25± 0.013 ^b	0.37± 0.013 ^b	0.43± 0.025 ^b	0.10± 0.006 ^a	0.29± 0.006 ^b	0.22± 0.006 ^b	0.25± 0.006 ^b
KS 244+	0.14± 0.006 ^a	0.26± 0.006 ^b	0.32± 0.022 ^b	0.37± 0.025 ^b	0.09± 0.011 ^a	0.16± 0.006 ^b	0.21± 0.011 ^b	0.22± 0.013 ^b
BN 101	0.11± 0.006 ^a	0.23± 0.011 ^b	0.33± 0.006 ^b	0.46± 0.006 ^b	0.09± 0.011 ^a	0.20± 0.013 ^b	0.29± 0.013 ^b	0.31± 0.013 ^b
30V92	0.19± 0.018 ^a	0.2± 0.013 ^a	0.25± 0.013 ^a	0.21± 0.011 ^a	0.06± 0.006 ^a	0.11± 0.006 ^b	0.14± 0.006 ^b	0.07± 0.006 ^b
BN 1133	0.14± 0.013 ^a	0.22± 0.017 ^b	0.27± 0.006 ^b	0.12± 0.050 ^a	0.075± 0.011 ^a	0.11± 0.022 ^b	0.21± 0.011 ^b	0.07± 0.006 ^a
DHANYA	0.09± 0.011 ^a	0.19± 0.006 ^b	0.22± 0.017 ^b	0.12± 0.050 ^a	0.075± 0.011 ^a	0.15± 0.011 ^b	0.16± 0.006 ^b	0.08± 0.006 ^a
SWARNA	0.13± 0.011 ^a	0.21± 0.013 ^b	0.23± 0.006 ^b	0.16± 0.006 ^a	0.06± 0.006 ^a	0.16± 0.006 ^b	0.18± 0.006 ^b	0.08± 0.006 ^a

LEAF: CD- Treatments: 0.08; Cultivars: 0.10 ROOT: CD- Treatments: 0.05; Cultivars: 0.07
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 13B: ANOVA of data presented in Table 13 (Proline content in root)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	0.255522	3	0.085174	37.2833	6.23E-08	3.159908
Columns	0.083943	6	0.013991	6.124098	0.001227	2.661305
Error	0.041121	18	0.002285			
Total	0.380586	27				

Table 14A: ANOVA of data presented in Table 14 (Proline content of leaf)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	0.087995	3	0.029332	6.204098	0.004412	3.159908
Columns	0.077478	6	0.012913	2.731279	0.045752	2.661305
Error	0.0851	18	0.004728			
Total	0.250573	27				

Table 14B: ANOVA of data presented in Table 14 (Proline content of root)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.054219	3	0.018073	8.850159	0.000806	3.159908
Columns	0.065642	6	0.01094	5.357371	0.0025	2.661305
Error	0.036758	18	0.002042			
Total	0.156618	27				

Table 15: Total chlorophyll, chlorophyll-a and chlorophyll-b content in the leaves of maize cultivars during drought stress *in vitro*

CULTIVARS	Treatment No. of Days	Total chlorophyll (mg/g tissue FW)	Chlorophyll-a (mg/g tissue FW)	Chlorophyll-b (mg/g tissue FW)
KS 244	0	3.56±0.038 ^a	2.50±0.013 ^a	1.06±0.045 ^a
	3	2.25±0.031 ^b	1.28±0.032 ^b	0.97±0.056 ^b
	5	1.68±0.025 ^b	1.22±0.023 ^b	0.46±0.032 ^b
	7	1.15±0.018 ^b	0.97±0.015 ^b	0.18±0.009 ^b
KS 244+	0	3.48±0.028 ^a	2.45±0.009 ^a	1.03±0.035 ^a
	3	2.43±0.028 ^b	1.49±0.015 ^a	0.94±0.015 ^a
	5	1.97±0.018 ^b	1.28±0.012 ^a	0.70±0.006 ^b
	7	1.36±0.023 ^b	1.14±0.006 ^a	0.22±0.015 ^b
BN 101	0	3.15±0.030 ^a	2.24±0.003 ^a	0.91±0.027 ^a
	3	2.31±0.024 ^b	1.53±0.013 ^b	0.78±0.013 ^b
	5	1.65±0.032 ^b	1.21±0.026 ^b	0.44±0.009 ^b
	7	1.03±0.032 ^b	0.76±0.012 ^b	0.26±0.023 ^b
30V92	0	3.28±0.027 ^a	2.20±0.007 ^a	1.08±0.029 ^a
	3	2.84±0.023 ^b	2.11±0.029 ^b	0.88±0.003 ^b
	5	2.36±0.018 ^b	1.61±0.003 ^b	0.75±0.015 ^b
	7	1.28±0.031 ^b	0.96±0.015 ^b	0.31±0.019 ^b
BN 1133	0	2.33±0.035 ^a	1.57±0.019 ^a	0.76±0.021 ^a
	3	1.30±0.009 ^b	0.87±0.012 ^b	0.44±0.003 ^b
	5	1.31±0.015 ^b	0.78±0.009 ^b	0.53±0.017 ^b
	7	0.88±0.018 ^b	0.57±0.012 ^b	0.31±0.006 ^b
DHANYA	0	4.15±0.046 ^a	3.23±0.010 ^a	0.98±0.144 ^a
	3	2.12±0.018 ^b	1.38±0.024 ^b	0.74±0.031 ^b
	5	0.83±0.033 ^b	0.51±0.020 ^b	0.32±0.034 ^b
	7	0.67±0.006 ^b	0.42±0.012 ^b	0.25±0.017 ^b
SWARNA	0	3.16±0.032 ^a	1.83±0.028 ^a	1.33±0.009 ^a
	3	1.89±0.026 ^b	1.48±0.010 ^b	0.73±0.022 ^b
	5	1.38±0.006 ^b	0.87±0.012 ^b	0.51±0.012 ^b
	7	1.09±0.026 ^b	0.64±0.024 ^b	0.45±0.020 ^b

Total chlorophyll CD- Treatments: 0.42; Cultivars: 0.55

Chlorophyll-a: CD- Treatments: 0.39; Cultivars: 0.51

Chlorophyll-b: CD- Treatments: 0.15; Cultivars: 0.21

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 16: Total chlorophyll, chlorophyll-a and chlorophyll-b content in the leaves maize cultivars during drought stress *in vivo*

CULTIVARS	Treatment No. of Days	Total chlorophyll (mg/g tissue FW)	Chlorophyll-a (mg/g tissue FW)	Chlorophyll-b (mg/g tissue FW)
KS 244	0	3.40±0.027 ^a	2.16±0.007 ^a	1.24±0.030 ^a
	3	3.91±0.040 ^b	2.70±0.027 ^b	1.23±0.009 ^a
	5	3.34±0.030 ^a	2.64±0.040 ^b	1.70±0.007 ^b
	7	2.70±0.030 ^b	2.33±0.010 ^b	1.35±0.059 ^b
KS 244+	0	2.64±0.012 ^a	1.75±0.029 ^a	0.89±0.079 ^a
	3	3.42±0.032 ^b	2.41±0.038 ^b	1.00±0.003 ^b
	5	2.95±0.009 ^b	1.19±0.003 ^b	0.71±0.009 ^b
	7	1.50±0.019 ^b	1.09±0.010 ^b	0.42±0.006 ^b
BN 101	0	3.44±0.046 ^a	2.80±0.041 ^a	0.64±0.040 ^a
	3	3.75±0.110 ^a	2.53±0.120 ^a	1.22±0.023 ^a
	5	2.01±0.042 ^b	1.32±0.017 ^b	0.69±0.026 ^a
	7	1.73±0.019 ^b	1.21±0.006 ^b	0.52±0.023 ^a
30V92	0	3.72±0.017 ^a	2.61±0.001 ^a	1.11±0.012 ^a
	3	3.04±0.007 ^b	1.96±0.006 ^a	1.07±0.012 ^a
	5	1.70±0.018 ^b	0.88±0.014 ^b	0.83±0.026 ^b
	7	0.70±0.070 ^b	0.51±0.032 ^b	0.19±0.047 ^b
BN 1133	0	3.25±0.012 ^a	2.10±0.003 ^a	1.15±0.012 ^a
	3	2.91±0.035 ^b	2.04±0.070 ^a	0.87±0.049 ^b
	5	1.51±0.022 ^b	0.90±0.010 ^b	0.61±0.017 ^b
	7	0.56±0.026 ^b	0.47±0.007 ^b	0.088±0.31 ^b
DHANYA	0	3.38±0.055 ^a	2.87±0.096 ^a	0.53±0.045 ^a
	3	2.68±0.024 ^b	2.37±0.020 ^b	0.31±0.009 ^b
	5	1.71±0.032 ^b	1.47±0.013 ^b	0.24±0.037 ^b
	7	0.52±0.003 ^b	0.43±0.015 ^b	0.094±0.013 ^b
SWARNA	0	3.47±0.064 ^a	1.77±0.021 ^a	1.70±0.066 ^a
	3	2.59±0.022 ^b	1.69±0.012 ^b	0.90±0.030 ^b
	5	1.47±0.043 ^b	1.31±0.022 ^b	0.17±0.038 ^b
	7	0.76±0.018 ^b	0.62±0.022 ^b	0.13±0.023 ^b

Total chlorophyll CD- Treatments: 0.55; Cultivars: 0.73

Chlorophyll-a: CD- Treatments: 0.48; Cultivars: 0.63

Chlorophyll-b: CD- Treatments: 0.29; Cultivars: 0.39

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 17: Total chlorophyll, chlorophyll-a and chlorophyll-b content in the leaves of maize cultivars during flooding stress

CULTIVARS	Treatment No. of Days	Total chlorophyll (mg/g tissue FW)	Chlorophyll-a (mg/g tissue FW)	Chlorophyll-b (mg/g tissue FW)
KS 244	0	1.88±0.002 ^a	1.14±0.017 ^a	0.74±0.018 ^a
	3	1.64±0.011 ^b	0.96±0.015 ^b	0.68±0.025 ^a
	5	1.18±0.012 ^b	0.67±0.029 ^b	0.51±0.027 ^b
	7	1.10±0.018 ^b	0.66±0.009 ^b	0.44±0.022 ^b
KS 244+	0	1.85±0.029 ^a	1.11±0.030 ^a	0.74±0.037 ^a
	3	1.65±0.020 ^b	0.97±0.010 ^b	0.68±0.021 ^a
	5	1.14±0.012 ^b	0.65±0.021 ^b	0.49±0.030 ^b
	7	1.06±0.016 ^b	0.62±0.029 ^b	0.44±0.033 ^b
BN 101	0	1.82±0.031 ^a	1.09±0.016 ^a	0.73±0.023 ^a
	3	1.52±0.075 ^b	0.94±0.038 ^b	0.58±0.021 ^b
	5	1.20±0.031 ^b	0.87±0.014 ^b	0.33±0.015 ^b
	7	1.11±0.062 ^b	0.80±0.017 ^b	0.31±0.044 ^b
30V92	0	1.50±0.020 ^a	0.86±0.007 ^a	0.64±0.013 ^a
	3	1.28±0.008 ^b	0.77±0.003 ^b	0.51±0.005 ^b
	5	0.82±0.039 ^b	0.56±0.031 ^b	0.26±0.019 ^b
	7	0.62±0.024 ^b	0.37±0.006 ^b	0.25±0.023 ^b
BN 1133	0	1.95±0.012 ^a	1.02±0.005 ^a	0.93±0.017 ^a
	3	1.74±0.008 ^b	0.94±0.012 ^b	0.80 ±0.009 ^b
	5	0.82±0.039 ^b	0.56±0.031 ^b	0.26±0.019 ^b
	7	0.62±0.024 ^b	0.37±0.006 ^b	0.25±0.023 ^b
DHANYA	0	1.74±0.027 ^a	0.94±0.007 ^a	0.80±0.033 ^b
	3	1.28±0.015 ^b	0.77±0.021 ^b	0.51±0.009 ^b
	5	0.76±0.015 ^b	0.53±0.013 ^b	0.22±0.003 ^b
	7	0.55±0.014 ^b	0.37±0.016 ^b	0.18±0.030 ^b
SWARNA	0	1.78±0.028 ^a	0.98±0.008 ^a	0.79±0.033 ^b
	3	1.42±0.020 ^b	0.89±0.010 ^b	0.53±0.021 ^b
	5	0.92±0.024 ^b	0.57±0.008 ^b	0.35±0.023 ^b
	7	0.67±0.018 ^b	0.40 ±0.015 ^b	0.27±0.010 ^b

Total chlorophyll CD- Treatments: 0.14; Cultivars: 0.19

Chlorophyll-a: CD- Treatments: 0.08; Cultivars: 0.11

Chlorophyll-b: CD- Treatments: 0.09; Cultivars: 0.12

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 15A: ANOVA of data presented in Table 15 (Total chlorophyll)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	19.25765	3	6.419215	46.67962	1.08E-08	3.159908
Columns	2.498294	6	0.416382	3.027874	0.031613	2.661305
Error	2.475296	18	0.137516			
Total	24.23124	27				

Table 15B: ANOVA of data presented in Table 15 (Chlorophyll-a)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	9.011475	3	3.003825	25.07208	1.2E-06	3.159908
Columns	1.566343	6	0.261057	2.17897	0.093647	2.661305
Error	2.156537	18	0.119808			
Total	12.73435	27				

Table 15C: ANOVA of data presented in Table 15 (Chlorophyll-b)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	2.115715	3	0.705238	38.21171	5.16E-08	3.159908
Columns	0.225775	6	0.037629	2.038845	0.112882	2.661305
Error	0.33221	18	0.018456			
Total	2.6737	27				

Table 16A: ANOVA of data presented in Table 16 (Total chlorophyll)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	21.37946	3	7.126487	29.2576	3.88E-07	3.159908
Columns	5.279758	6	0.87996	3.61265	0.015752	2.661305
Error	4.384392	18	0.243577			
Total	31.04361	27				

Table 16B: ANOVA of data presented in Table 16 (Chlorophyll-a)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	9.114154	3	3.038051	16.65637	1.97E-05	3.159908
Columns	3.705711	6	0.617619	3.386145	0.020527	2.661305
Error	3.283124	18	0.182396			
Total	16.10299	27				

Table 16C: ANOVA of data presented in Table 16 (Chlorophyll-b)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	2.714542	3	0.904847	13.40778	7.72E-05	3.159908
Columns	0.865104	6	0.144184	2.136479	0.099086	2.661305
Error	1.214761	18	0.067487			
Total	4.794406	27				

Table 17A: ANOVA of data presented in Table 17 (Total chlorophyll)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	4.293912	3	1.431304	89.36155	5.29E-11	3.159908
Columns	0.656438	6	0.109406	6.830641	0.000665	2.661305
Error	0.288306	18	0.016017			
Total	5.238656	27				

Table 17B: ANOVA of data presented in Table 17 (chlorophyll-a)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	1.139677	3	0.379892	75.93392	2.06E-10	3.159908
Columns	0.293078	6	0.048846	9.763551	7.49E-05	2.661305
Error	0.090053	18	0.005003			
Total	1.522808	27				

Table 17C: ANOVA of data presented in Table 17 (Chlorophyll-b)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	1.020757	3	0.340252	48.32688	8.18E-09	3.159908
Columns	0.126251	6	0.021042	2.988612	0.033177	2.661305
Error	0.126732	18	0.007041			
Total	1.273739	27				

4.8. Changes in antioxidant enzymes in maize cultivars subjected to drought and flooding

4.8.1. *In vitro* drought

The activity of antioxidative enzymes like peroxidase (POX), catalase (CAT), ascorbate peroxidase (APOX), superoxide dismutase (SOD) and glutathione reductase (GR) during *in vitro* drought stress showed various types of trends in the leaves of different test cultivars. POX (Fig 15A) activity in KS 244, KS 244+ and BN101 showed a slight decrease in activity on the 3rd day of drought stress, but again the activity increased on the 7th day of stress. In the other cultivars the activity declined drastically on the 7th day of stress. CAT (Fig 15B) and GR (Fig 15C) showed a similar trend in KS 244, KS 244+ and BN 101 where a significant increase in activities was observed during drought stress. But in case of 30V92, BN 1133, Dhanya and Swarna a steep decline in their activities was observed during the period of stress. APOX (Fig 15D) and SOD (Fig 15E) activities in all the seven cultivars increased in the initial stages of stress but during prolonged stress a decrease in the activity of these antioxidative enzymes was observed.

4.8.2. *In vivo* drought

POX activity in the leaves of seven cultivars in case of *in vivo* drought stress showed different trend in different cultivars (Fig 16A). In KS 244+, KS 244 and BN 101 POX activity increased significantly with drought stress. But in BN 1133, 30V92, Swarna and Dhanya the activity decreased on the 7th day of stress. APOX activity (Fig 16B) increased in the initial period of stress and then decreased after the 7th day in all the cultivars. Similar type of trend was seen in case of SOD (Fig 16C) also, where the activity decreased after the 5th day of stress in all the seven cultivars. GR (Fig 16D) and CAT (Fig 16E) activity in KS 244, KS 244+ and BN 101 increased with the increase in drought stress but in the other four cultivars i.e BN 1133, 30V92, Swarna

and Dhanya the GR and CAT activity showed very insignificant increase during the initial stages of drought with a decrease in the later stage of stress.

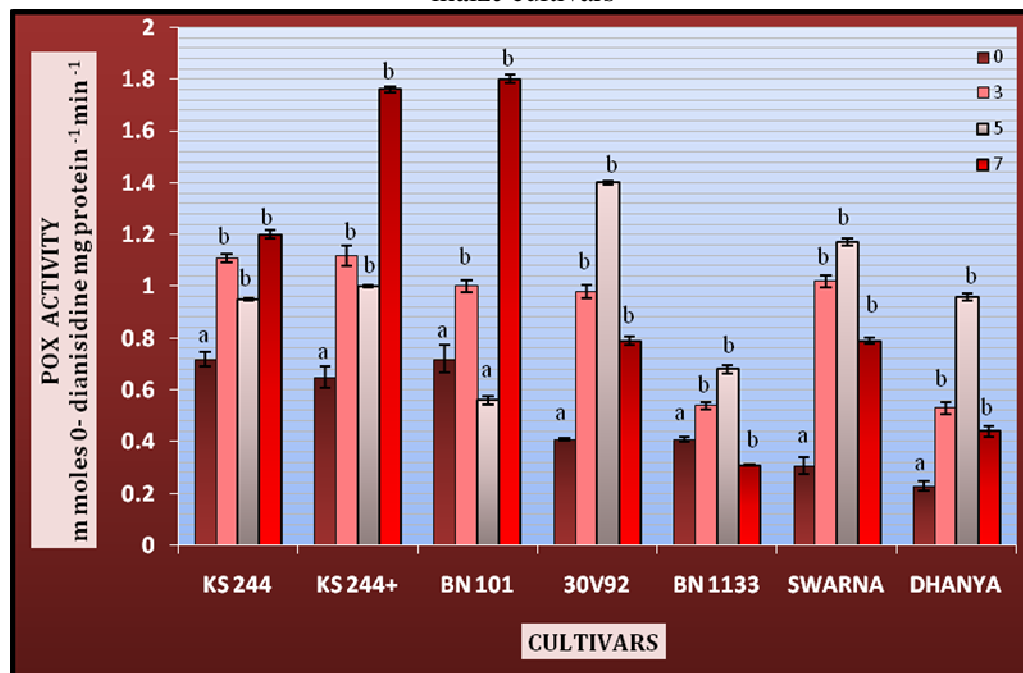
4.8.3. Flooding stress

During flooding stress antioxidative enzymes like POX, CAT, APOX, SOD and GR showed different trends in their activity in the leaves of different test cultivars. POX activity (Fig 17A) showed an increasing trend in the activity in the initial days of flooding stress but gradually with prolonged stress the activity decreased in all the test cultivars. In KS 244, KS 244+ and BN 101 the activity decreased on the 7th day of stress and in BN1133, 30V92, Swarna and Dhanya the activity decreased from the 5th day of stress. CAT activity (Fig 17B) showed a gradual, significant increase in KS 244, KS 244+ and BN 101. But in BN 1133, 30V92, Swarna and Dhanya the catalase activity decreased during the period of stress. APOX activity (Fig 17C) and SOD activity (Fig 17D) in BN 101, KS 244, KS 244+ showed an increase in activity till the 10th day of flooding stress but a decrease in the activity was seen on the 7th day of stress a different trend was seen in case of BN 1133, Swarna, Dhanya and 30V92 where the APOX activity showed an initial increase and then decreased in activity after the 3rd day stress. The GR activity (Fig 17E) in KS 244, KS 244+ and BN 101 showed a significant increase in activity during the period of flooding stress. But like APOX and SOD, the GR activity showed a same type of trend in BN 1133, Swarna, Dhanya and 30V92 during flooding stress.

4.9. Isozyme analysis of antioxidative enzymes under water stress

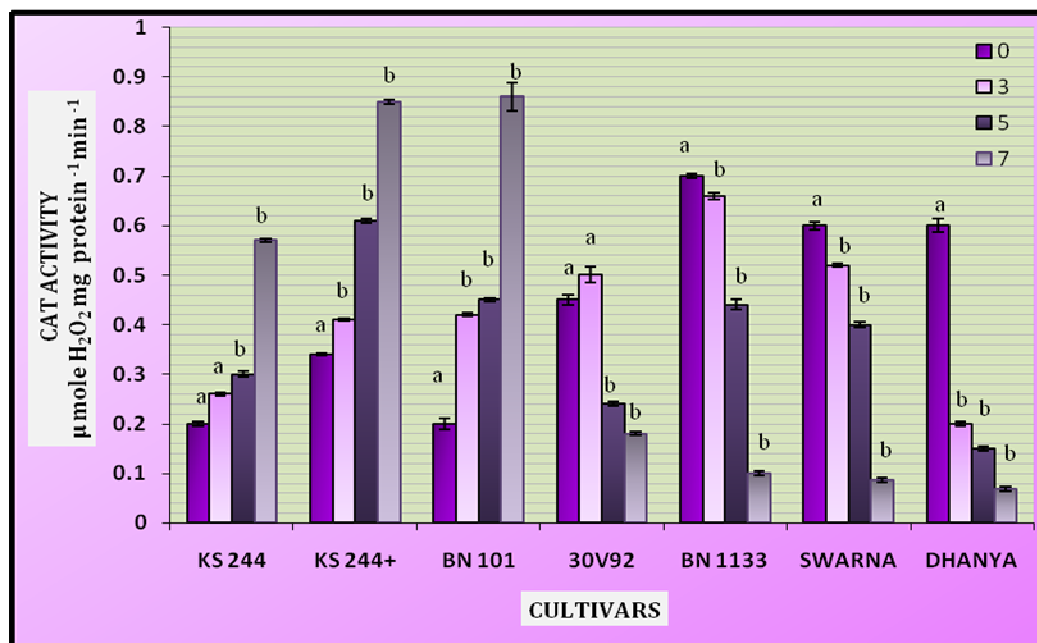
Experiments were performed in the leaves of different cultivars of maize to determine whether water stress (drought and flooding) could induce new isozyme of peroxidase and catalase. Analysis of isozyme was done on native PAGE and the bands were visualized using suitable chemical dyes mentioned in the material and methods. In the plants subjected to water stress many new isozyme bands were observed in the test cultivars of maize when compared to the control plants.

Fig 15A: Peroxidase activity during drought stress *in vitro* in the leaves of seven maize cultivars



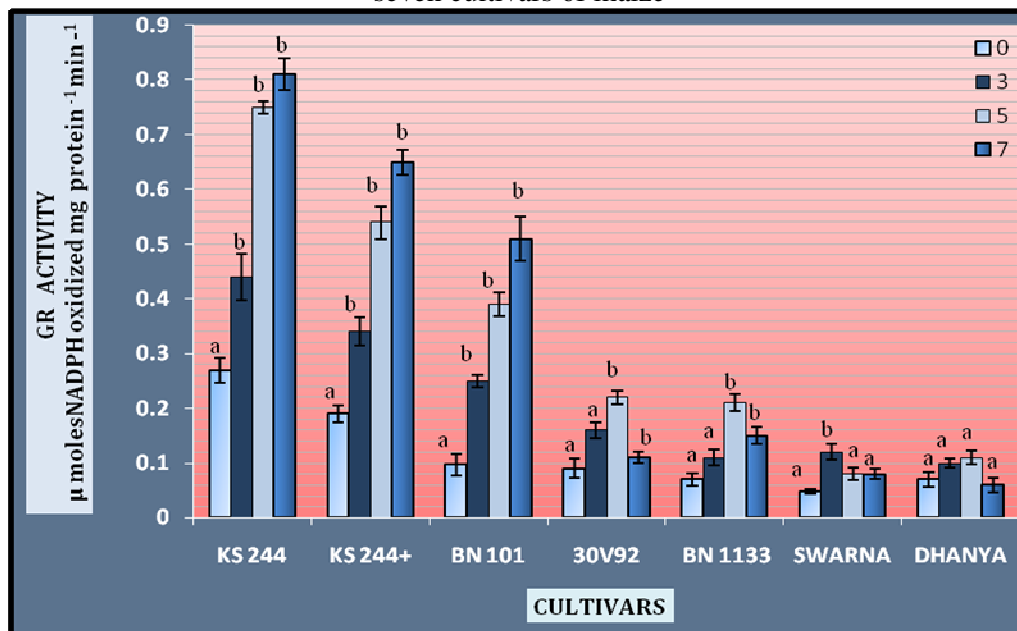
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 15B: Catalase activity during drought stress *in vitro* in the leaves of seven maize cultivars



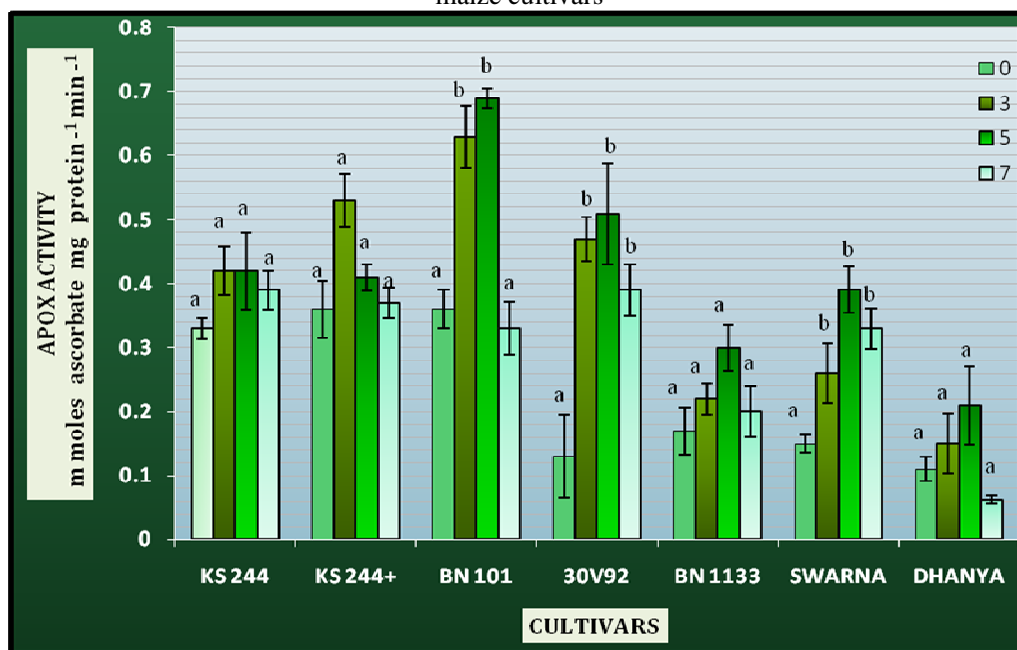
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 15C: Glutathione reductase activity during drought stress *in vitro* in the leaves of seven cultivars of maize



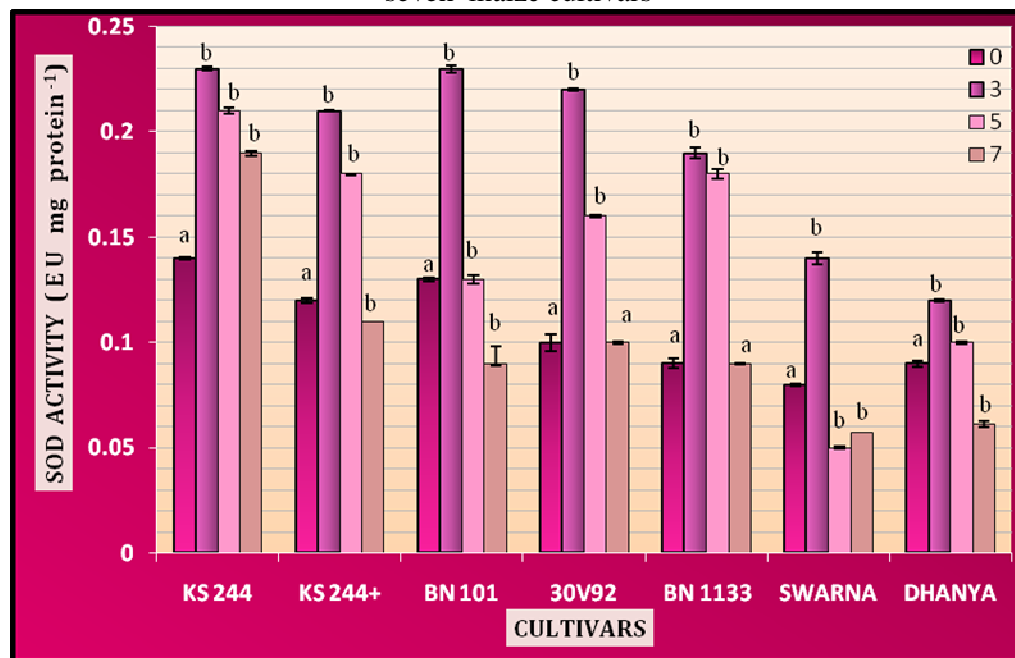
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 15D: Ascorbate peroxidase activity during drought stress *in vitro* in the leaves of seven maize cultivars



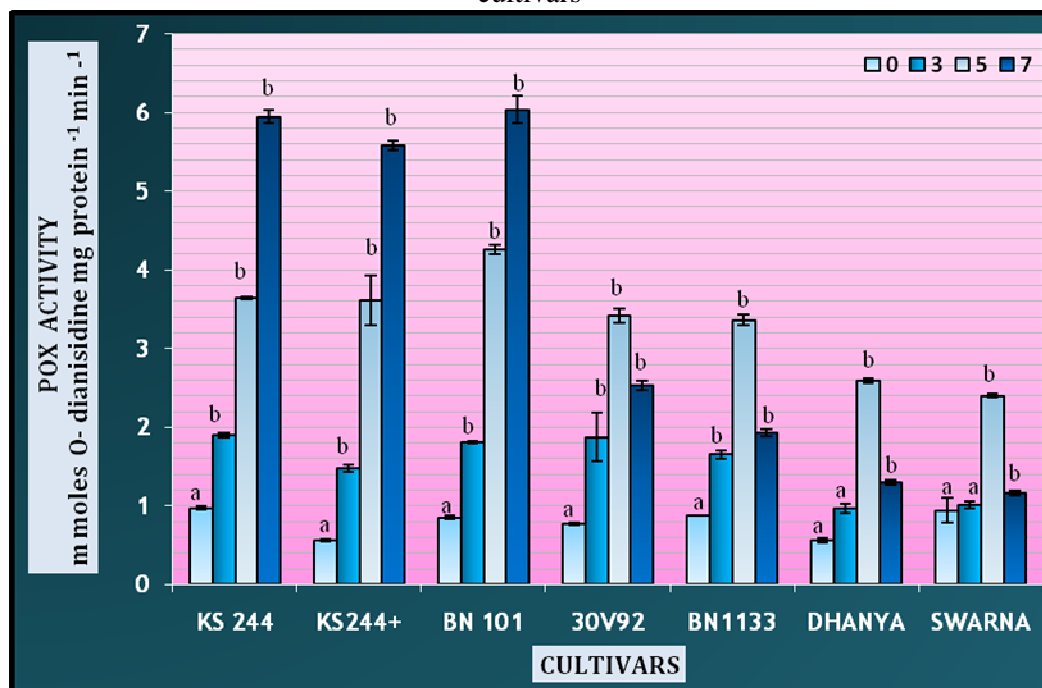
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 15E:Superoxide dismutase activity during drought stress *in vitro* in the leaves of seven maize cultivars



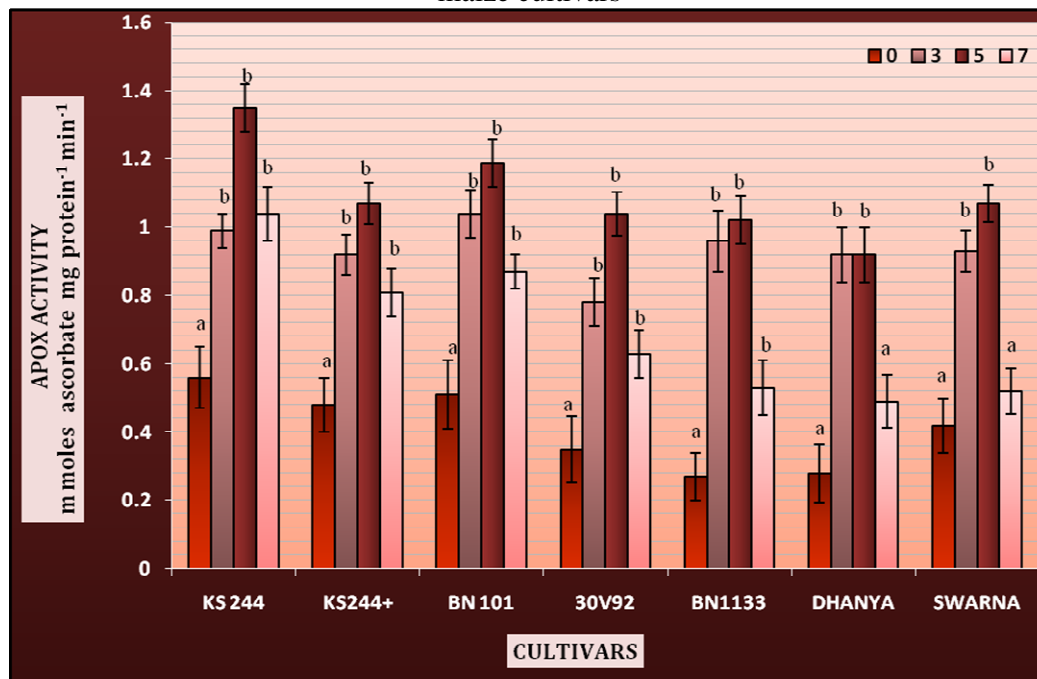
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 16A:Peroxidase activity during drought stress *in vivo* in the leaves of seven maize cultivars



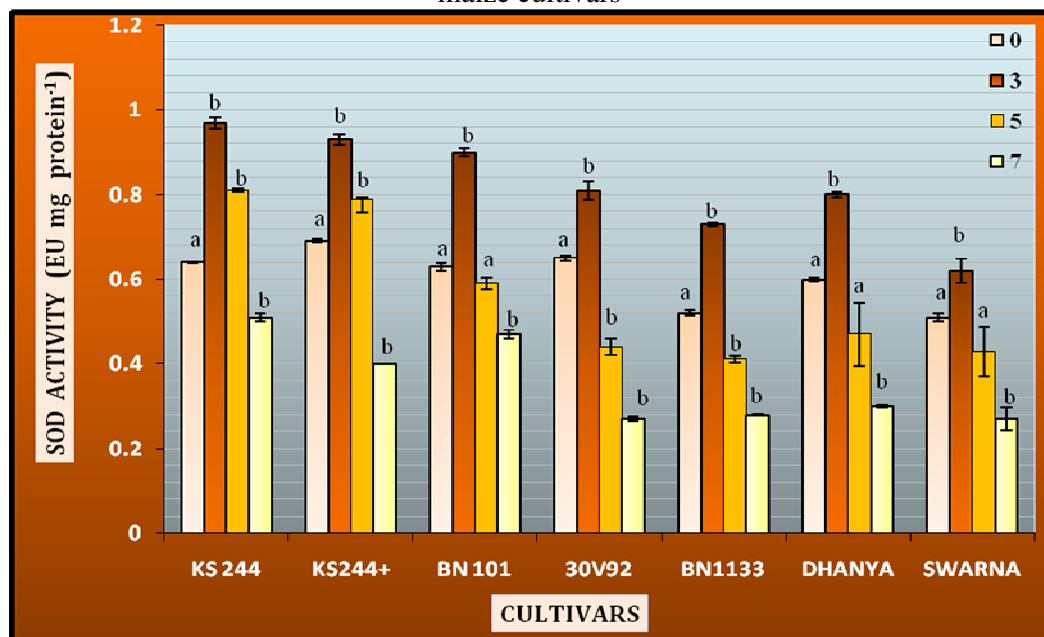
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 16B: Ascorbate peroxidase activity during drought stress *in vivo* in the leaves of maize cultivars



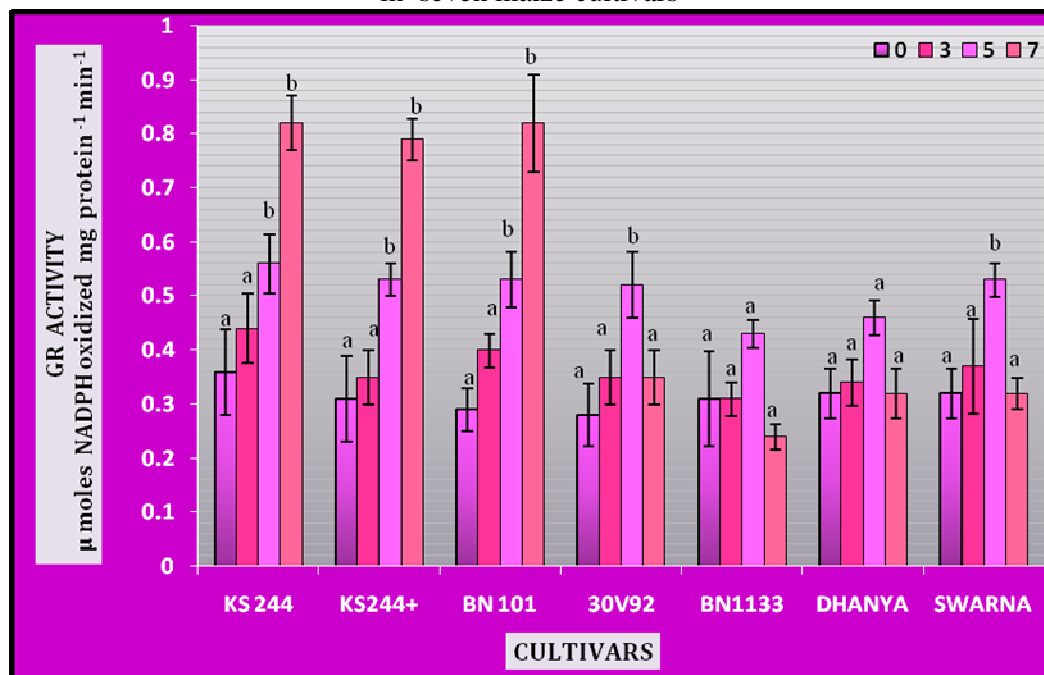
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 16C: Superoxide dismutase activity during drought stress *in vivo* in the leaves of maize cultivars



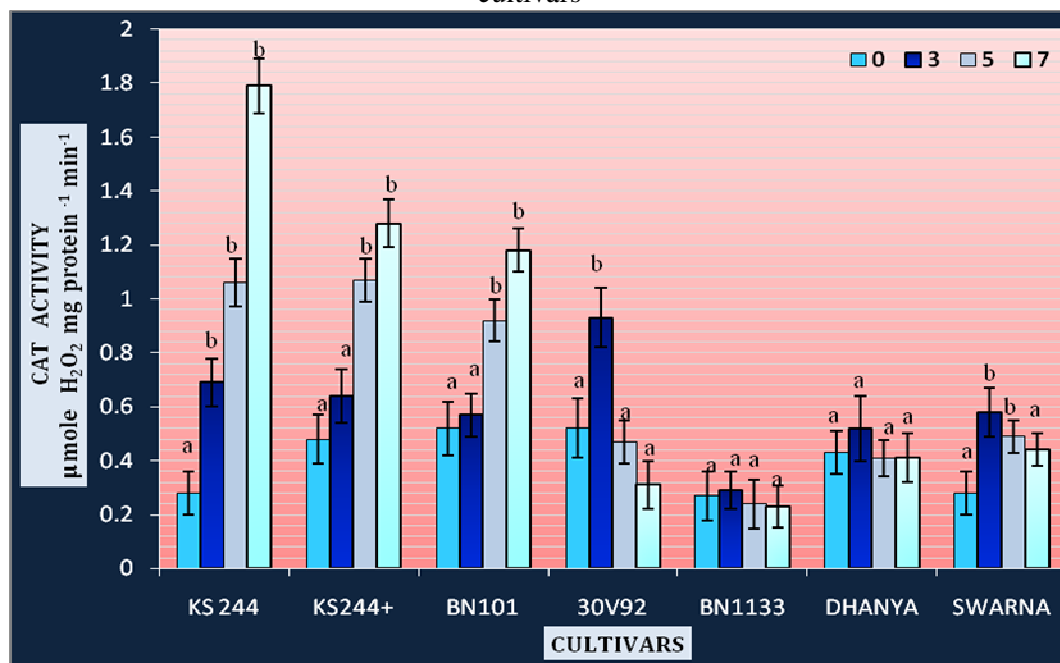
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 16D: Glutathione reductase activity during drought stress *in vivo* in the leaves of in seven maize cultivars



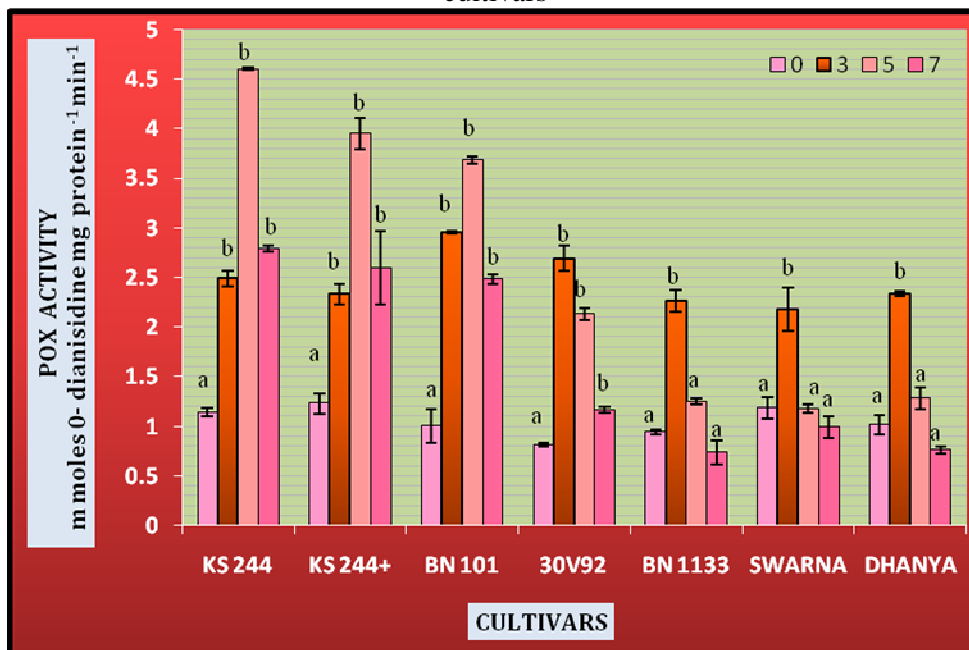
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 16E: Catalase activity during drought stress *in vivo* in the leaves of seven maize cultivars



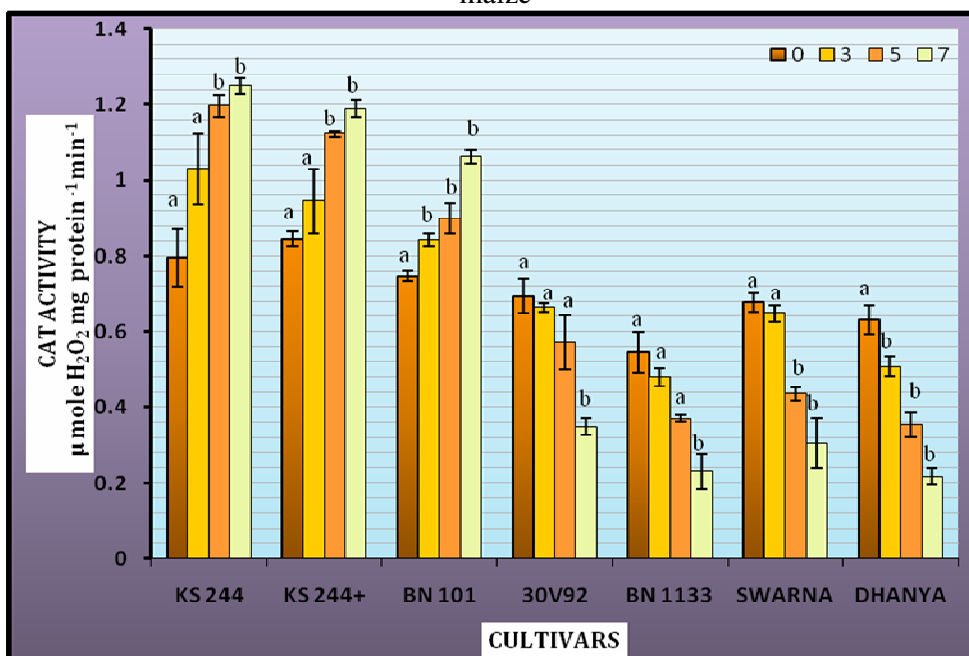
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 17A: Peroxidase activity during flooding stress in the leaves of seven maize cultivars



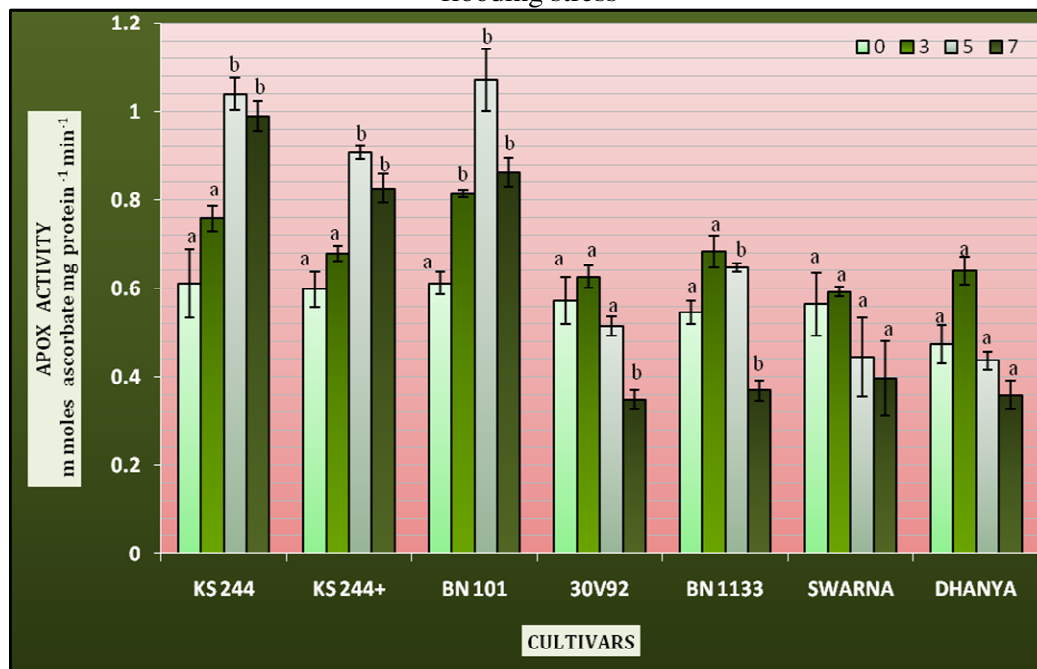
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 17B: Catalase activity during flooding stress in the leaves of seven cultivars of maize



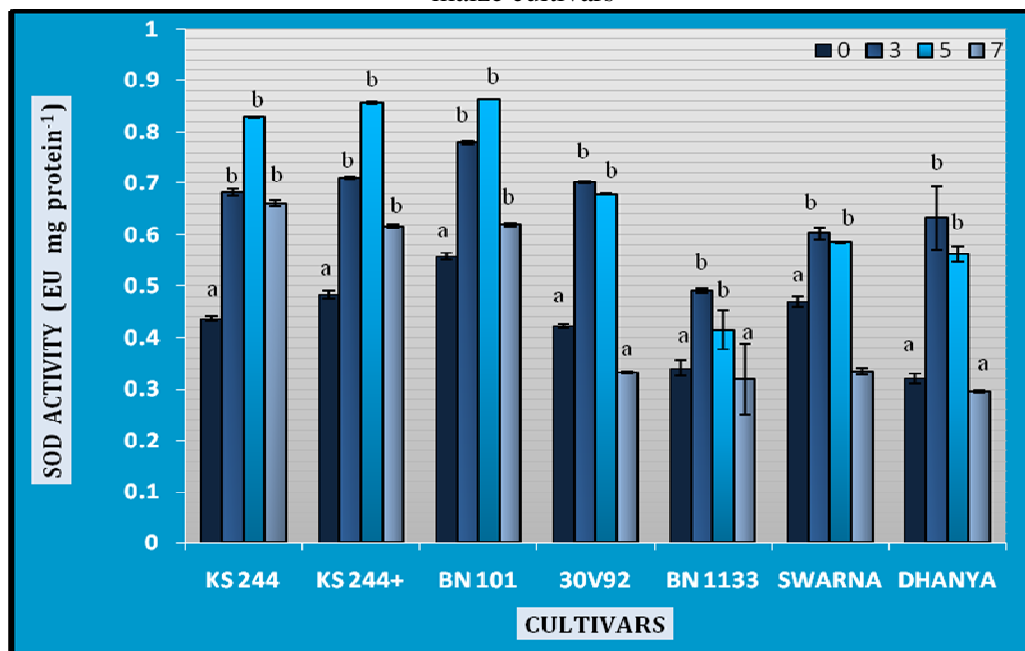
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 17C: Ascorbate peroxidase activity in the leaves of maize cultivars during flooding stress



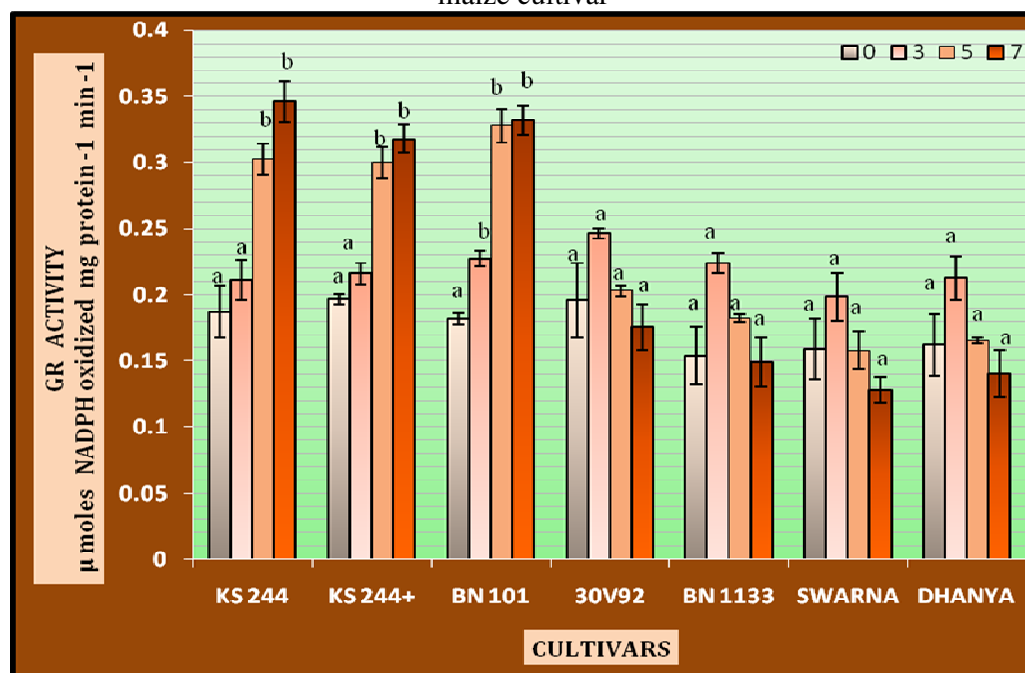
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 17D: Superoxide dismutase activity during flooding stress in the leaves of seven maize cultivars



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 17E: Glutathione reductase activity during flooding stress in the leaves of seven maize cultivar



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

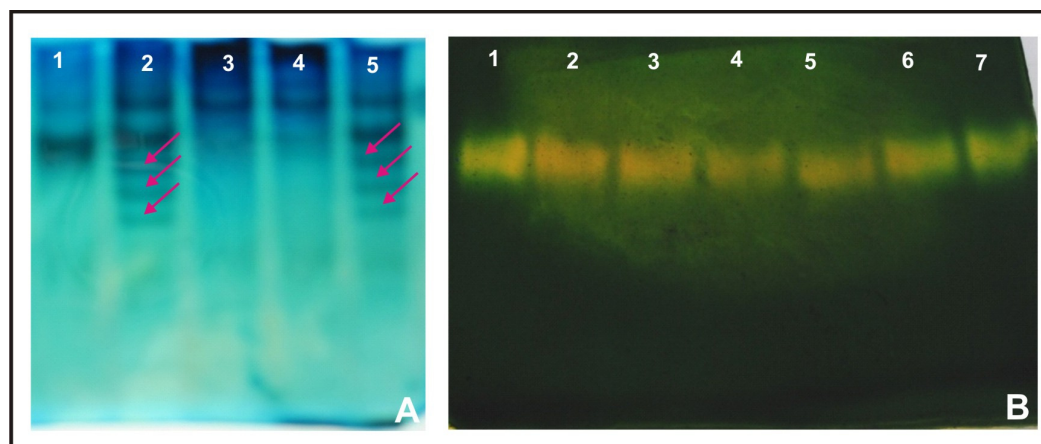


Fig 18: Analysis of peroxidase and catalase isozyme in the leaves of of KS 244 cultivar during drought stress *in vivo*. **A: Peroxidase isozyme** -Lane 1: 0-day, Lane 2: 7-day, Lane 3 and 4: 3-day, Lane 5:7-day of drought stress. **B: Catalase isozyme**- Lane 1&7:0-day, Lane 2&3:7-day, Lane 4&5: 5-day, Lane 6&7: 3-day

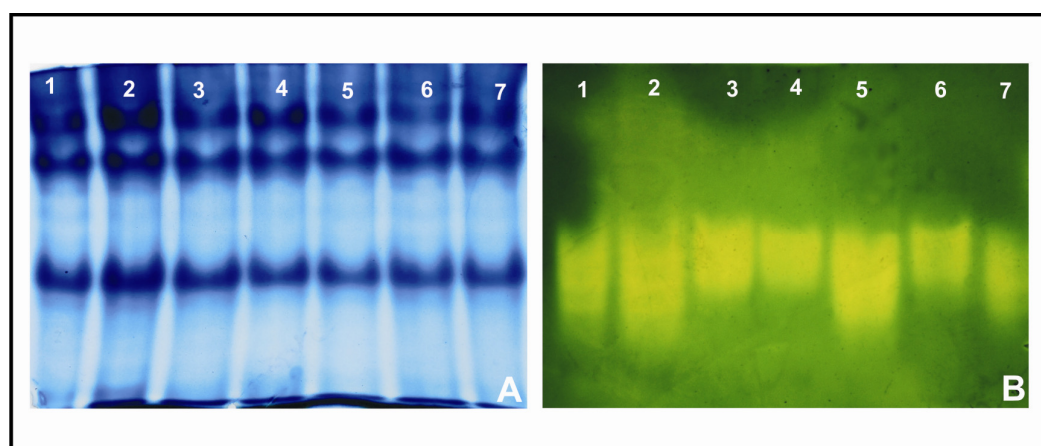


Fig 19: Analysis of peroxidase and catalase isozyme in the leaves of Swarna cultivar during drought stress *in vivo*. **A: Peroxidase isozyme** -Lane 1: 0-day, Lane 2: 3day, Lane 3: 5day, Lane 4:7-day, Lane 5: 0-day, Lane 6: 3-day, Lane 7: -5-day **B: Catalase isozyme**-Lane 1:0-day, Lane 2: 7-day, Lane 3: 5-day, Lane 4: 3-day,Lane 5: 7-day, Lane 6: 5-day, Lane 7: 0-day

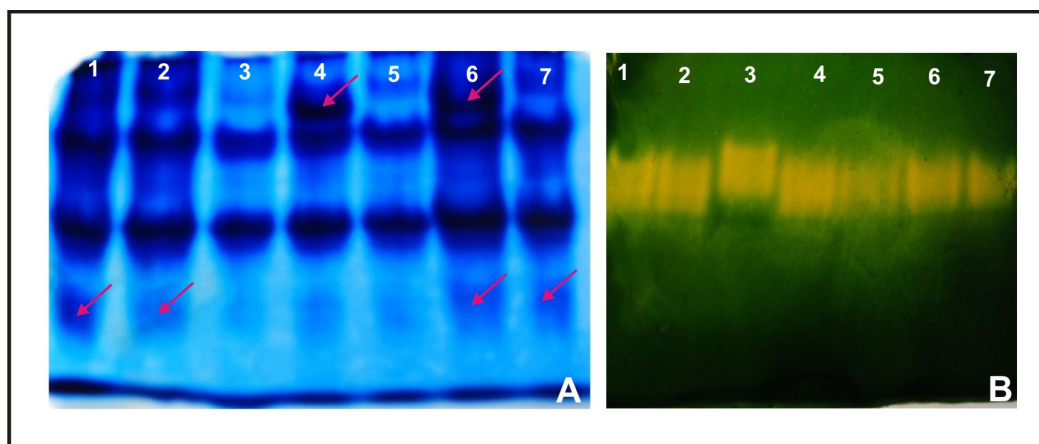


Fig 20: Analysis of peroxidase and catalase isozyme in the leaves of KS 244+ cultivar during *in vivo* drought stress and flooding stress. **A: Peroxidase isozyme** - Lane 1: 0-day, Lane 2: 3-day drought, Lane 3: 5-day drought, Lane 4: 7-day drought, Lane 5: 7-day flooding, Lane 6: 5-day flooding, Lane 7: 3-day flooding **B: Catalase isozyme** - Lane 1: 0-day, Lane 2: 3-day drought, Lane 3: 5-day drought, Lane 4: 7-day drought, Lane 5: 7-day flooding, Lane 6: 5-day flooding, Lane 7: 3-day flooding

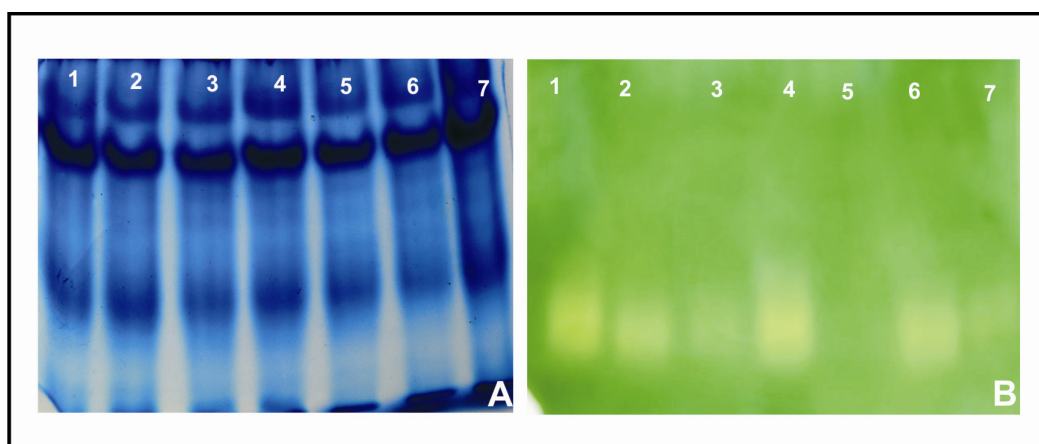


Fig 21: Analysis of peroxidase and catalase isozyme in the leaves of Dhanya cultivar during *in vivo* drought stress. **A: Peroxidase isozyme** - Lane 1&7: 0-day, Lane 2&6: 3-day, Lane 3&5: 5-day, Lane 4: 7-day **B: Catalase isozyme** - Lane 1: 0-day, Lane 2: 3-day, Lane 3: 5-day, Lane 4: 7-day, Lane 6: 3-day

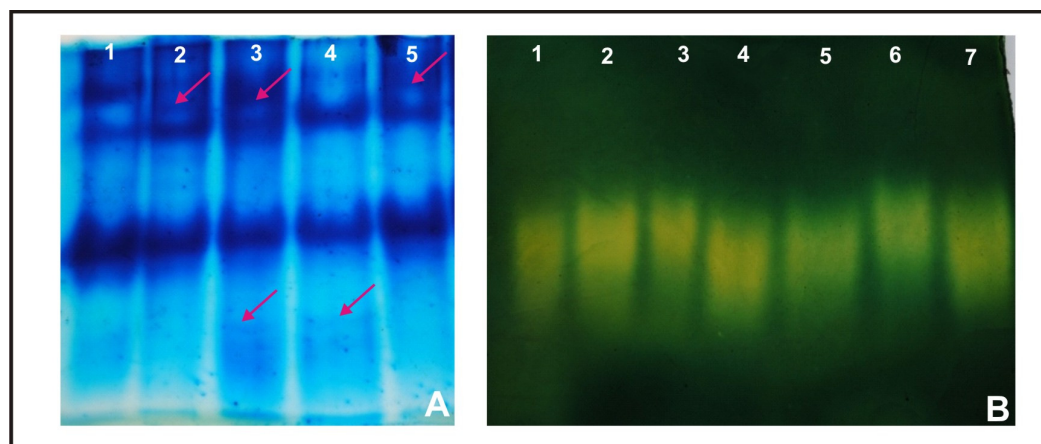


Fig 22: Analysis of peroxidase and catalase isozyme in the leaves of 30V92 cultivar during *in vivo* drought stress. **A: Peroxidase isozyme** –Lane 1: 3-day, Lane 2: 5-day, Lane 3: 7-day, Lane 4: 0-day, Lane 5: 5-day **B: Catalase isozyme**– Lane 1: 0-day, Lane 2: 3-day drought, Lane 3: 5-day drought, Lane 4: 7-day drought, Lane 5: 7-day flooding, Lane 6: 5-day flooding, Lane 7: 3-day flooding.

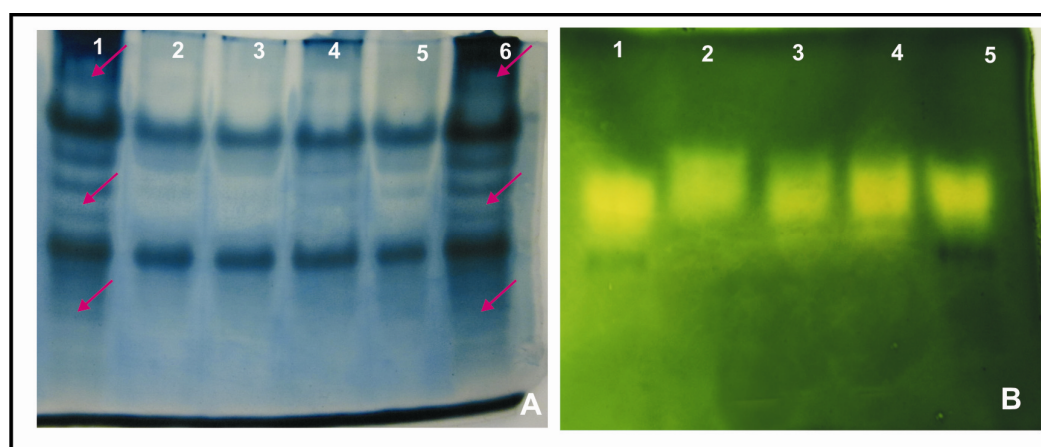


Fig 23: Analysis of peroxidase and catalase isozyme in the leaves of BN 101 cultivar during *in vivo* drought stress. **A: Peroxidase isozyme** –Lane 1&6: 7-day, Lane 2&5: 5-day, Lane 3: 0-day, Lane 4: 3-day **B: Catalase isozyme** - Lane 1&5: 7-day, Lane 2: 5-day, Lane 3: 3-day, Lane 4: 0-day

Table 18: Relative mobility (R_m) value of peroxidase isozyme in the leaves of maize cultivars during *in vivo* water stress.

Cultivar	Treatment	Lane Number	No. of isozyme bands	R_m value
KS 244	0-day drought	1	2	0.17,0.29
	7-day drought	2	5	0.17,0.29,0.36,0.41,0.48
	3-day drought	3	3	0.12,0.17,0.29
	5-day drought	4	3	0.12,0.17,0.29
	7-day drought	5	5	0.17,0.29,0.36,0.41,0.48
SWARNA	0-day drought	1	6	0.09,0.18,0.28,0.63,0.72
	3-day drought	2	6	0.09,0.18,0.28,0.63,0.72
	5-day drought	3	6	0.09,0.18,0.28,0.63,0.72
	7-day drought	4	6	0.09,0.18,0.28,0.63,0.72
	3-day flooding	5	6	0.09,0.18,0.28,0.63,0.72
	5-day flooding	6	6	0.09,0.18,0.28,0.63,0.72
	7-day flooding	7	6	0.09,0.18,0.28,0.63,0.72
KS 244+	0-day drought	1	5	0.13,0.34,0.55,0.47,0.68
	3-day drought	2	5	0.13,0.34,0.55,0.47,0.68
	5-day drought	3	4	0.13,0.34,0.55,0.47
	7-day drought	4	5	0.13,0.17,0.34,0.55,0.47
	7-day flooding	5	4	0.13,0.34,0.55,0.47
	5-day flooding	6	6	0.13,0.17,0.34,0.55,0.47,0.68
	3-day flooding	7	5	0.13,0.34,0.55,0.47,0.68
DHANYA	0-day drought	1	6	0.16,0.26,0.40,0.50,0.60,0.74
	3-day drought	2	6	0.16,0.26,0.40,0.50,0.60,0.74
	5-day drought	3	6	0.16,0.26,0.40,0.50,0.60,0.74
	7-day drought	4	6	0.16,0.26,0.40,0.50,0.60,0.74
	5-day drought	5	6	0.16,0.26,0.40,0.50,0.60,0.74
	3-day drought	6	6	0.16,0.26,0.40,0.50,0.60,0.74
	7-day drought	7	6	0.16,0.26,0.40,0.50,0.60,0.74
30V92	3-day drought	1	4	0.14,0.24,0.54,0.64
	5-day drought	2	5	0.14,0.20,0.24,0.54,0.64
	7-day drought	3	6	0.20,0.34,0.54,0.64,0.78
	0-day drought	4	4	0.14,0.24,0.54,0.78
	5-day drought	5	5	0.14,0.20,0.24,0.54,0.64

Table19A: Relative mobility (R_m) value of catalase isozyme in leaves of maize cultivars during *in vivo* water stress.

Cultivar	Treatment	Lane Number	No. of isozyme bands	R_m value
KS 244	0-day drought	1	1	0.25
	0-day drought	2	1	0.25
	3-day drought	3	1	0.29
	3-day drought	4	1	0.29
	5-day drought	5	1	0.30
	7-day drought	6	1	0.27
	0-day drought	7	1	0.25
SWARNA	0-day drought	1	1	0.49
	3-day drought	2	1	0.46
	5-day drought	3	1	0.44
	7-day drought	4	1	0.47
	3-day flooding	5	1	0.44
	5-day flooding	6	1	0.44
	7-day flooding	7	1	0.47
KS 244+	0-day drought	1	1	0.30
	3-day drought	2	1	0.31
	5-day drought	3	1	0.26
	7-day drought	4	1	0.30
	7-day flooding	5	1	0.31
	5-day flooding	6	1	0.30
	3-day flooding	7	1	0.31
DHANYA	0-day drought	1	1	0.72
	3-day drought	2	1	0.76
	5-day drought	3	1	0.72
	7-day drought	4	1	0.74
	3-day drought	6	1	0.76
30V92	0-day drought	1	1	0.44
	3-day drought	2	1	0.40
	5-day drought	3	1	0.42
	7-day drought	4	1	0.46
	7-day flooding	5	1	0.42
	5-day flooding	6	1	0.38
	3-day flooding	7	1	0.40

Table 18B: Relative mobility (R_m) value of peroxidase isozyme in the leaves of maize cultivars during *in vivo* water stress.

Cultivar	Treatment	Lane Number	No. of isozyme bands	R_m value
BN 101	7-day drought	1	6	0.10,0.20,0.30,0.46,0.54,0.72
	5-day drought	2	4	0.20,0.30,0.40,0.54
	0-day drought	3	4	0.20,0.30,0.40,0.54
	3-day drought	4	4	0.20,0.30,0.40,0.54
	5-day drought	5	4	0.20,0.30,0.40,0.54
	7-day drought	6	6	0.10,0.20,0.30,0.46,0.54,0.72

Table19A: Relative mobility (R_m) value of catalase isozyme in the leaves of maize cultivars during *in vivo* water stress

Cultivar	Treatment	Lane Number	No. of isozyme bands	R_m value
BN 101	7-day drought	1	1	0.35
	5-day drought	2	1	0.28
	3-day drought	3	1	0.28
	0-day drought	4	1	0.30
	7-day drought	5	1	0.35

4.10. Changes in small antioxidants during water stress

Water stress in seven cultivars of maize led to changes in various types of antioxidants like ascorbate, carotenoid and α -tocopherol.

4.10.1. Ascorbate

Drought stress in both *in vitro* (Table 20) and *in vivo* (Table 21) conditions led to an increase in ascorbate content in the leaves of all the seven cultivars of maize. In case of KS 244, KS 244+ and BN 101 the ascorbate content increase on the 7th day of drought stress was almost nine times greater than that of the control. In 30V92, BN 1133, Swarna and Dhanya the increase of ascorbate content was almost four times than that of the control. In flooding stress (Table 22) there was an increase in the ascorbate content in KS 244, KS 244+ and BN 101 during the period of stress but in the other cultivars -30V92, BN 1133, Dhanya and Swarna the ascorbate content increased till the 5th day of stress but after which a decrease in the ascorbate content was noticed on the 7th day of stress.

4.10.2. Carotenoids

In drought stress *in vitro* (Fig 24A) and *in vivo* (Fig 24B) conditions, the seven test cultivars of maize exhibited different trends. Cultivars KS 244, KS 244+, BN 101 showed an initial increase in the carotenoid content in the leaves but decreased after the prolongation of drought stress. In the other cultivars there was a decrease in the carotenoid content from the initiation of drought stress in the plants. During flooding stress (Fig 24C) there was a decrease in the carotenoid content in all the cultivars of stress from the initial period of stress.

4.11.3. α -Tocopherol

α -Tocopherol content in the leaves of seven cultivars of maize showed a very similar result like that of ascorbate, where there was an increase during drought stress both *in vitro* (Table 23) and *in vivo* (Table 24) in all the seven cultivars of maize. Maximum increase was seen in BN101, KS 244 and KS 244+. But again in flood stress a decrease was seen on the 7th day of stress in BN 1133, Swarna and Dhanya (Table 25).

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Table 20: Ascorbate content in the leaves of seven maize cultivars during drought stress *in vitro*

CULTIVARS	Ascorbate content (mg g ⁻¹ tissue FW)			
	0d	3d	5d	7d
KS 244	1.19±0.044 ^a	2.81±0.010 ^b	5.12±0.007 ^b	7.22±0.0107 ^b
KS 244+	1.17±0.007 ^a	2.77±0.017 ^b	5.33±0.014 ^b	7.30±0.014 ^b
BN 101	1.47±0.007 ^a	2.62±0.014 ^b	5.55±0.010 ^b	7.17±0.021 ^b
30V92	0.93±0.007 ^a	2.55±0.031 ^b	2.43±0.011 ^b	4.92±0.010 ^b
BN 1133	0.72±0.010 ^a	1.17±0.010 ^b	1.47±0.024 ^b	2.19±0.010 ^b
DHANYA	1.19±0.044 ^a	2.62±0.024 ^b	2.89±0.008 ^b	4.22±0.021 ^b
SWARNA	0.92±0.004 ^a	1.47±0.019 ^b	2.76±0.007 ^b	2.42±0.017 ^b

CD- Treatments: 3.40; Cultivars: 4.50 Average of three replicate experiments. $\pm$ =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 21: Ascorbate content in the leaves of seven maize cultivars during drought stress *in vivo*

CULTIVARS	Ascorbate content (mg g ⁻¹ tissue FW)			
	0d	3d	5d	7d
KS 244	1.87±0.004 ^a	2.87±0.007 ^b	5.20±0.010 ^b	9.09±0.570 ^b
KS 244+	1.38±0.004 ^a	3.05±0.211 ^b	4.96±0.010 ^b	9.16±0.377 ^b
BN 101	1.81±0.035 ^a	3.08±0.093 ^b	5.15±0.111 ^b	9.99±0.400 ^b
30V92	1.62±0.047 ^a	2.15±0.017 ^b	3.13±0.011 ^b	5.86±0.098 ^b
BN 1133	1.47±0.010 ^a	2.45±0.037 ^b	2.92±0.072 ^b	3.27±0.180 ^b
DHANYA	1.70±0.013 ^a	2.62±0.159 ^b	3.92±0.020 ^b	4.79±0.330 ^b
SWARNA	1.33±0.015 ^a	1.44±0.007 ^b	3.00±0.021 ^b	3.44±0.017 ^b

CD- Treatments: 1.40; Cultivars: 1.86 Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 22: Ascorbate content in the leaves of seven cultivars of maize during flooding stress

CULTIVARS	Ascorbate content (mg g ⁻¹ tissue FW)			
	0d	3d	5d	7d
KS 244	2.01±0.008 ^a	2.44±0.024 ^b	4.64±0.360 ^b	5.83±0.020 ^b
KS 244+	1.92±0.017 ^a	2.45±0.010 ^b	4.06±0.007 ^b	5.75±0.023 ^b
BN 101	1.89±0.082 ^a	2.52±0.014 ^b	4.09±0.010 ^b	6.27±0.267 ^b
30V92	1.61±0.020 ^a	2.92±0.013 ^b	4.29±0.021 ^b	2.23±0.029 ^b
BN 1133	1.59±0.014 ^a	3.04±0.010 ^b	4.69±0.007 ^b	2.03±0.007 ^b
SWARNA	1.58±0.020 ^a	2.92±0.010 ^b	4.23±0.021 ^b	1.41±0.004 ^b
DHANYA	1.38±0.004 ^a	2.48±0.034 ^b	4.51±0.231 ^b	1.53±0.007 ^b

CD- Treatments: 1.30; Cultivars: 1.72 Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 23: α -Tocopherol content in the leaves of maize cultivars during drought stress *in vitro*

CULTIVARS	α -Tocopherol content (mg g ⁻¹ tissue FW)			
	0d	3d	5d	7d
KS 244	0.51±0.062 ^a	1.29±0.021 ^b	3.47±0.021 ^b	4.13±0.005 ^b
KS 244+	0.59±0.021 ^a	1.24±0.025 ^b	3.49±0.012 ^b	4.15±0.002 ^b
BN 101	0.79±0.033 ^a	1.21±0.021 ^b	3.20±0.049 ^b	3.55±0.056 ^b
30V92	0.92±0.043 ^a	0.98±0.049 ^a	1.26±0.001 ^b	3.10 ±0.188 ^b
BN 1133	0.63±0.021 ^a	1.34±0.033 ^b	1.42±0.012 ^b	2.09±0.011 ^b
DHANYA	0.62±0.054 ^a	0.89±0.021 ^b	1.76±0.012 ^b	2.96±0.098 ^b
SWARNA	0.73±0.025 ^a	0.95±0.121 ^a	1.28±0.054 ^b	2.15±0.051 ^b

CD- Treatments: 0.65; Cultivars: 0.86 Average of three replicate experiments. $\pm$ =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 24: α -Tocopherol content in the leaves of maize cultivars during drought stress *in vivo*

CULTIVARS	α -Tocopherol content (mg g ⁻¹ tissue FW)			
	0d	3d	5d	7d
KS 244	0.48±0.111 ^a	1.06±0.118 ^b	3.20±0.012 ^b	4.59±0.078 ^b
KS 244+	0.59±0.021 ^a	0.87±0.025 ^b	2.36±0.021 ^b	3.94±0.177 ^b
BN 101	0.49±0.002 ^a	0.83±0.013 ^b	2.44±0.198 ^b	3.55±0.056 ^b
30V92	0.46±0.004 ^a	0.61±0.049 ^a	1.26±0.001 ^b	3.09±0.188 ^b
BN 1133	0.45±0.003 ^a	0.69±0.005 ^b	0.97±0.073 ^b	1.97±0.135 ^b
DHANYA	0.42±0.001 ^a	0.56±0.004 ^b	0.70±0.040 ^b	1.03±0.010 ^b
SWARNA	0.69±0.002 ^a	0.95±0.121 ^a	1.25±0.030 ^b	2.15±0.051 ^b

CD- Treatments: 0. 69; Cultivars: 0.91 Average of three replicate experiments. $\pm$ =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 25: α -Tocopherol content in the leaves of seven cultivars of maize during flooding stress

CULTIVARS	α -Tocopherol content (mg g ⁻¹ tissue FW)			
	0d	3d	5d	7d
KS 244	0.97±0.012 ^a	1.45±0.033 ^b	2.06±0.033 ^b	2.28±0.012 ^b
KS 244+	0.87±0.025 ^a	1.33±0.056 ^b	2.36±0.021 ^b	2.88±0.043 ^b
BN 101	0.83±0.012 ^a	1.69±0.044 ^b	2.18±0.021 ^b	2.59±0.074 ^b
30V92	0.75±0.033 ^a	1.24±0.033 ^b	1.68±0.033 ^b	1.70±0.021 ^b
BN 1133	0.76±0.054 ^a	1.18±0.021 ^b	1.66±0.093 ^b	1.64±0.012 ^b
SWARNA	0.95±0.012 ^a	1.19±0.021 ^b	1.55±0.043 ^b	1.05±0.012 ^b
DHANYA	0.86±0.033 ^a	1.19±0.012 ^b	1.42±0.012 ^b	0.89±0.021 ^b

CD- Treatments: 0.38; Cultivars: 0.50 Average of three replicate experiments. $\pm$ =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 20A: ANOVA of data presented in Table 20

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	1954.754	3	651.5846	71.02142	3.59E-10	3.159908
Columns	942.8098	6	157.135	17.1274	1.5E-06	2.661305
Error	165.1406	18	9.17448			
Total	3062.704	27				

Table 21A: ANOVA of data presented in Table 21

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	96.30136	3	32.10045	20.51635	4.89E-06	3.159908
Columns	33.28764	6	5.54794	3.545852	0.01702	2.661305
Error	28.16331	18	1.564628			
Total	157.7523	27				

Table 22A: ANOVA of data presented in Table 22

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	27.64922	3	9.216408	6.90162	0.002733	3.159908
Columns	7.254414	6	1.209069	0.9054	0.512703	2.661305
Error	24.03716	18	1.335398			
Total	58.9408	27				

Table 23A: ANOVA of data presented in Table 23

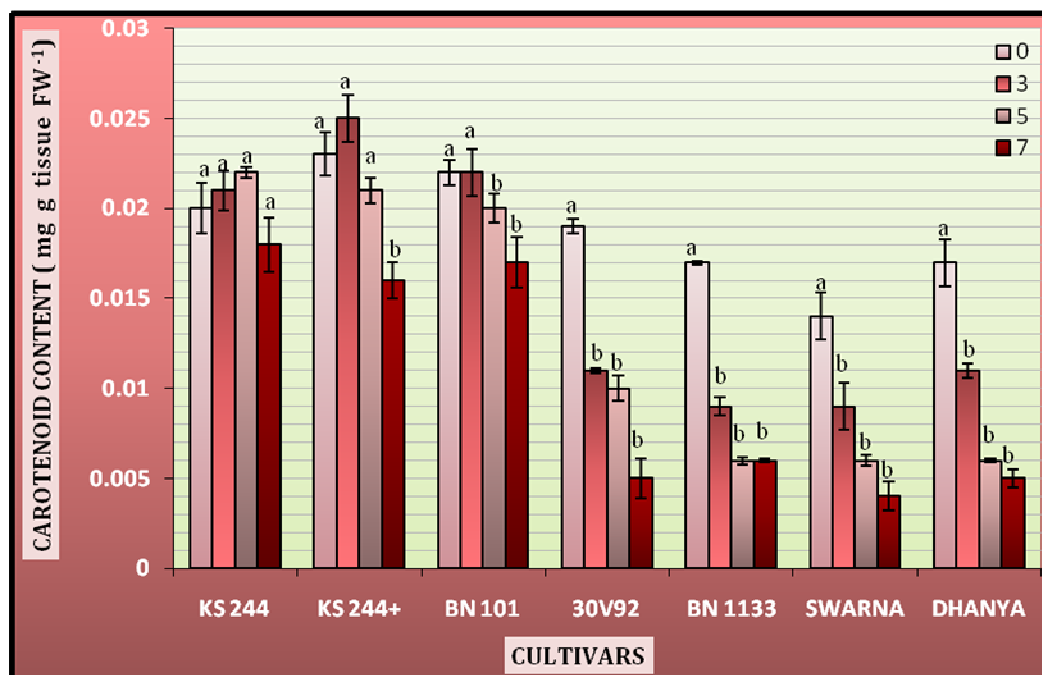
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	26.34483	3	8.781609	26.40656	8.22E-07	3.159908
Columns	5.394507	6	0.899084	2.703574	0.047385	2.661305
Error	5.985973	18	0.332554			
Total	37.72531	27				

Table 24A: ANOVA of data presented in Table 24

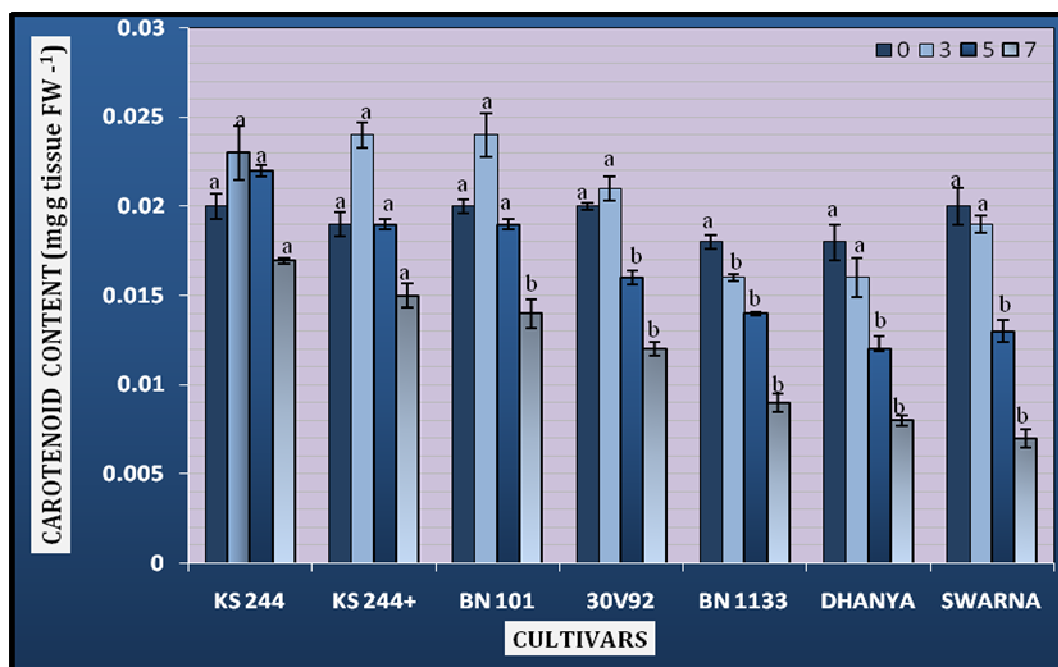
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	24.5036	3	8.167866	21.6182	3.41E-06	3.159908
Columns	7.905075	6	1.317512	3.48711	0.018228	2.661305
Error	6.800825	18	0.377824			
Total	39.2095	27				

Table 25A: ANOVA of data presented in Table 25

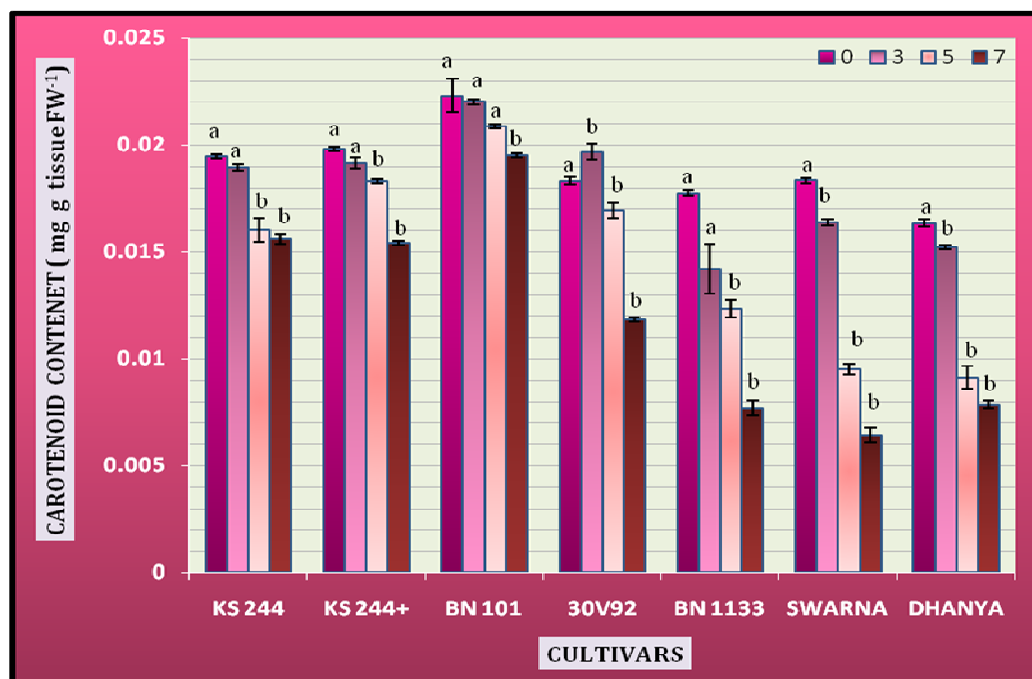
<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	4.86744	3	1.62248	14.04857	5.8E-05	3.159908
Columns	2.359184	6	0.393197	3.404579	0.020084	2.661305
Error	2.078833	18	0.115491			
Total	9.305457	27				

Fig 24A: Carotenoid content in the leaves of maize during drought stress *in vitro*

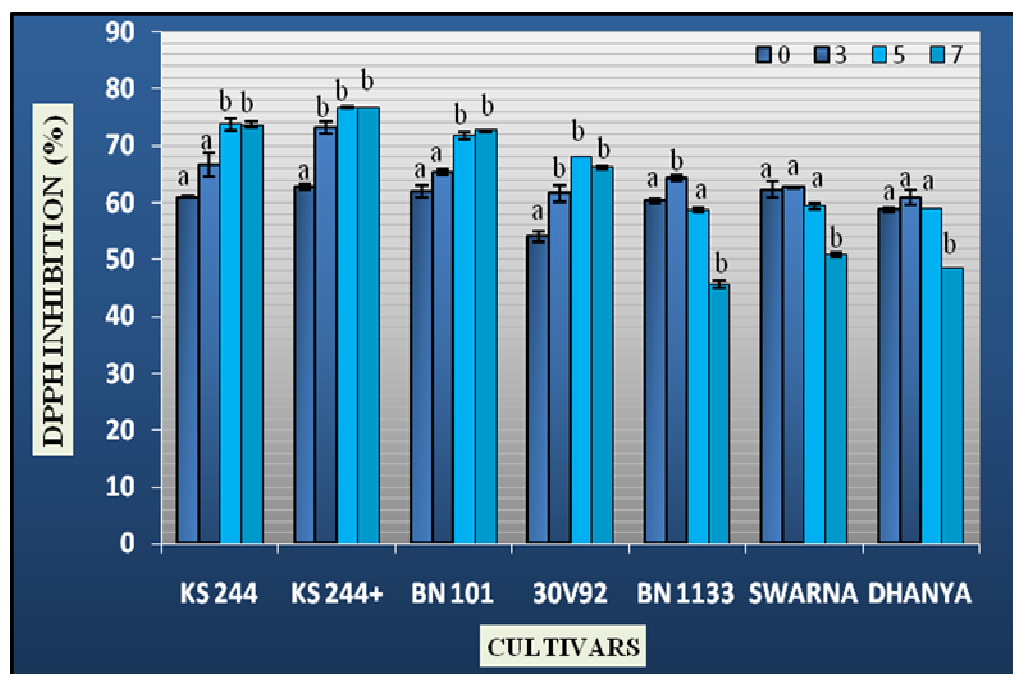
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 24B: Carotenoid content in the leaves of maize during drought stress *in vivo*

Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 24C: Carotenoid content in the leaves of maize cultivars during flooding stress

Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 25A: Total antioxidant activity in the leaves of maize cultivars during drought stress *in vitro*

Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

4.11. Effect of drought and flooding stress on total antioxidant activity in maize

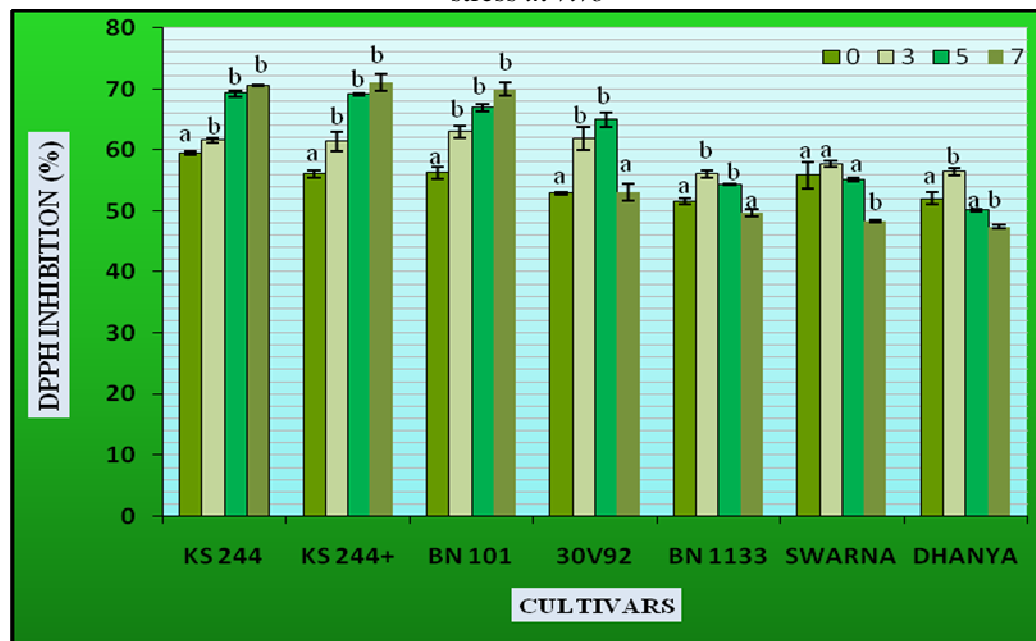
The total antioxidant activity in the leaves of the test plants was determined with respect to percent of DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) inhibition. During *in vitro* drought stress (Fig 25A) and *in vivo* drought stress (Fig 25B) it was seen that in cultivar KS 244, KS 244+ and BN 101, there was an increase in the DPPH inhibition during the period of stress. In case of 30V92 a decrease was seen on the 7th day of stress and in BN 1133, Dhanya and Swarna there was a slight increase on the 3rd day of stress but a significant decrease was seen on the 5th and the 7th day of water stress. In flooding stress (Fig 25C) KS 244, KS 244+, 30V92 and BN 101 increased during the period of stress but showed a slight decrease in the DPPH inhibition on the 7th day of stress. The other cultivars showed a significant decrease in the DPPH inhibition during the period of stress.

4.12. Effects of hydrogen peroxide accumulation in maize subjected to water stress

4.12.1. Hydrogen content

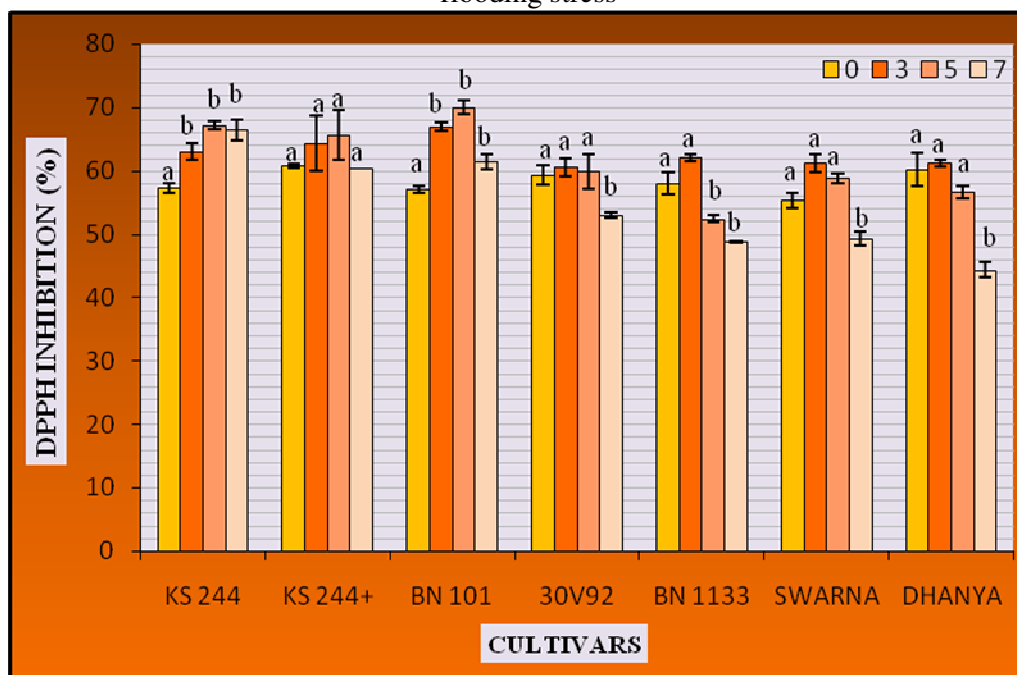
H₂O₂ was seen to accumulate in the leaves of all the cultivars during water stress in comparison to the control. During *in vitro* (Table 26) and *in vivo* (Fig 26) drought stress, maximum accumulation was seen in Dhanya, Swarna and BN 1333, which increased to almost 80-90 folds on the 7th day of stress. Not much increase was seen in KS 244, KS 244+ and BN 101 during drought stress, and on the 7th day the H₂O₂ accumulation decreased in these cultivars. In case of flooding stress (Fig 27), the cultivars showed a similar trend in the H₂O₂ accumulation in the leaves. In KS 244 and KS 244+ there was hardly any changes in H₂O₂ accumulation on the 7th day of stress and in BN 101 there was a slight decrease in H₂O₂ accumulation on the 7th day of flooding stress. The ANOVA of H₂O₂ accumulation during drought stress, *in vitro* condition showed a significant difference among the seven test cultivars.

Fig 25B: Total antioxidant activity in the leaves of maize cultivars during drought stress *in vivo*



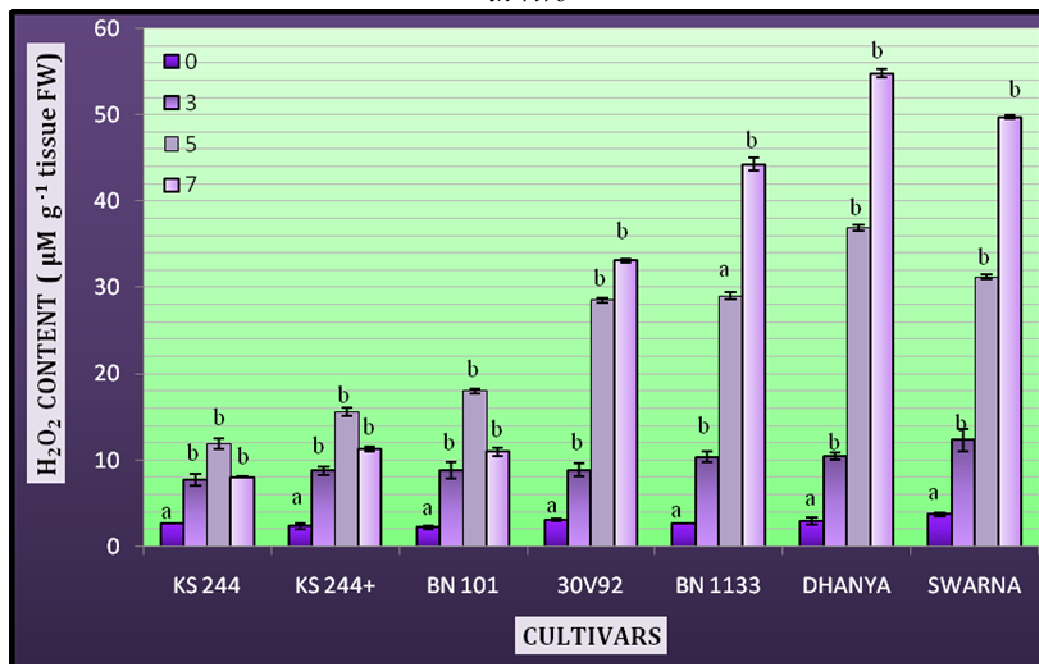
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Student's 't' test within each cultivar

Fig 25C: Total antioxidant activity in the leaves of seven maize cultivars during flooding stress



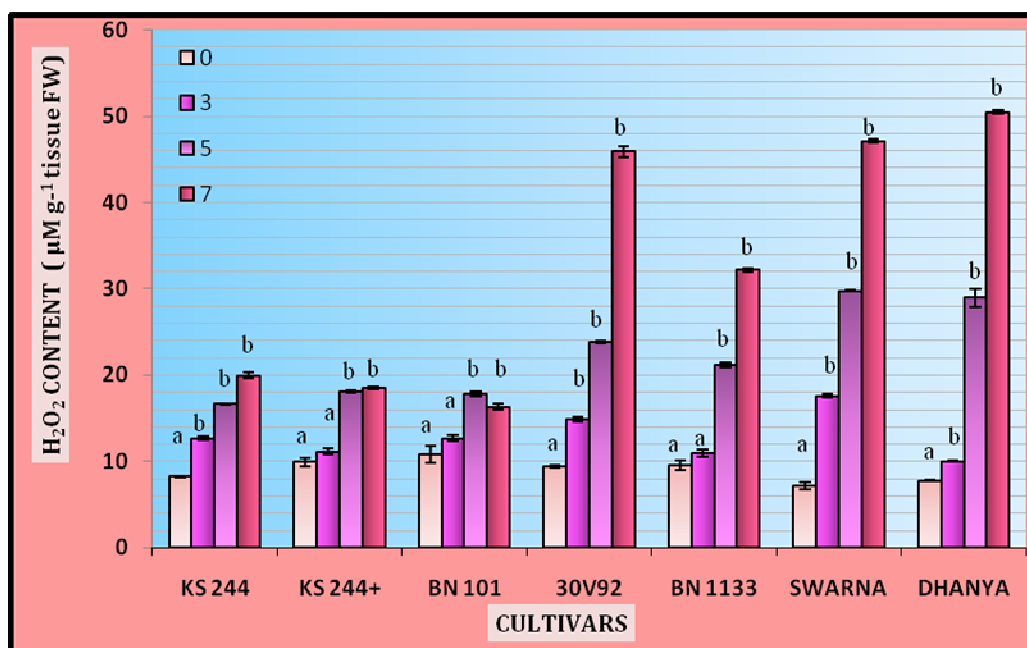
Different letters above different bars indicate the significant differences at $P \leq 0.05$ Student's 't' test within each cultivar

Fig 26: H₂O₂ content in leaves of seven cultivars of maize during drought stress *in vivo*



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 27: H₂O₂ content in leaves of seven cultivars of maize during flooding stress



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

4.12.2. Microscopic localization of H₂O₂

Accumulation of H₂O₂ in the leaves of maize cultivars was determined microscopically using DAB reagent as mentioned in Materials and Methods. It was observed that during drought stress in case of Dhanya, Swarna, BN 1333 there was greater accumulation of H₂O₂ on the 7th day of drought stress, as evident by darker staining of the cells in comparison to KS 244, KS 244+ and BN 101 where accumulation was lower even during prolonged period of drought (Fig 28A, 28B & 28C).

Table 26: H₂O₂ accumulation in the leaves of maize during *in vitro* drought stress

CULTIVARS	H ₂ O ₂ CONTENT($\mu\text{M g}^{-1}$ tissue FW)			
	0d	3d	5d	7d
KS 244	2.89±0.194 ^a	6.22±0.664 ^b	7.55±0.791 ^b	5.435±0.232 ^b
KS 244+	1.95±0.151 ^a	5.09±0.114 ^b	7.94±0.249 ^b	5.94±0.057 ^b
BN 101	1.87±0.802 ^a	4.19±0.342 ^a	6.24±0.292 ^b	5.25±0.347 ^b
30V92	6.40±0.348 ^a	5.14±0.262 ^a	9.83±0.302 ^b	18.34±0.396 ^b
BN 1133	5.49±0.297 ^a	11.49±0.193 ^b	12.51±0.262 ^b	17.65±0.356 ^b
DHANYA	2.19±0.157 ^a	6.44±0.050 ^b	10.59±0.253 ^b	17.94±0.468 ^b
SWARNA	2.67±0.221 ^a	6.47±0.107 ^b	12.15±0.179 ^b	13.44±0.488 ^b

CD- Treatments: 3.09; Cultivars: 4.08 Average of three replicate experiments. $\pm$ =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 26A: ANOVA of data presented in Table 26

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	296.4182	3	98.80607	13.08132	8.97E-05	3.159908
Columns	187.4502	6	31.2417	4.13621	0.008749	2.661305
Error	135.9579	18	7.553219			
Total	619.8263	27				

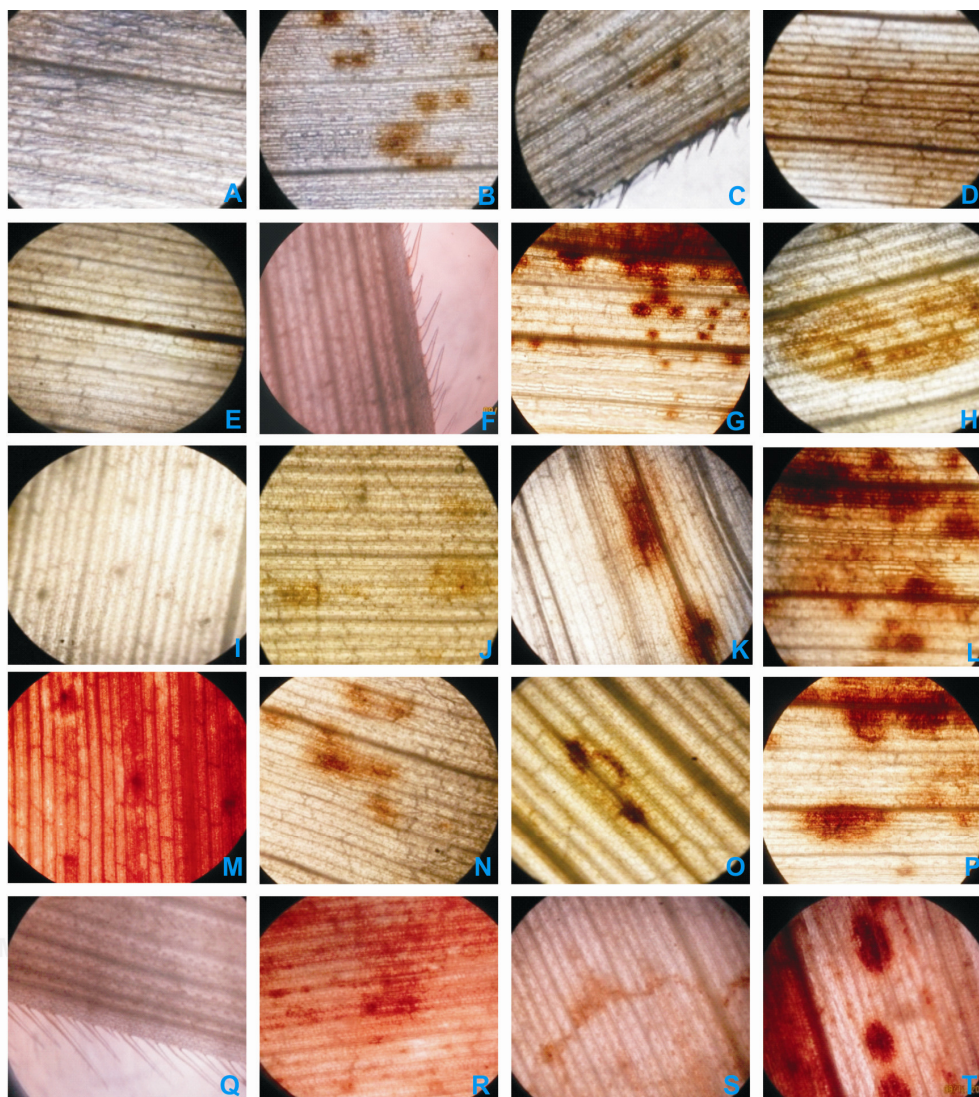


Fig 28A: Hydrogen peroxide localization by staining the leaves of maize subjected to drought stress *in vivo* with 3,3'Diaminobenzidine (DAB) and observing it in compound microscope (magnification 10X)

A,B,C,D: 0,3,5,7 day of drought stress in Swarna respectively **E,F,G,H:** 0,3,5,7 day of drought stress in BN 1133 respectively **I,J,K,L:** 0,3,5,7 day of drought stress in Dhanya respectively **M,N,O,P:** 0,3,5,7 day of drought stress in 30V92 respectively **Q,R,S,T:** 0,3,5,7 day of drought stress in BN 101 respectively

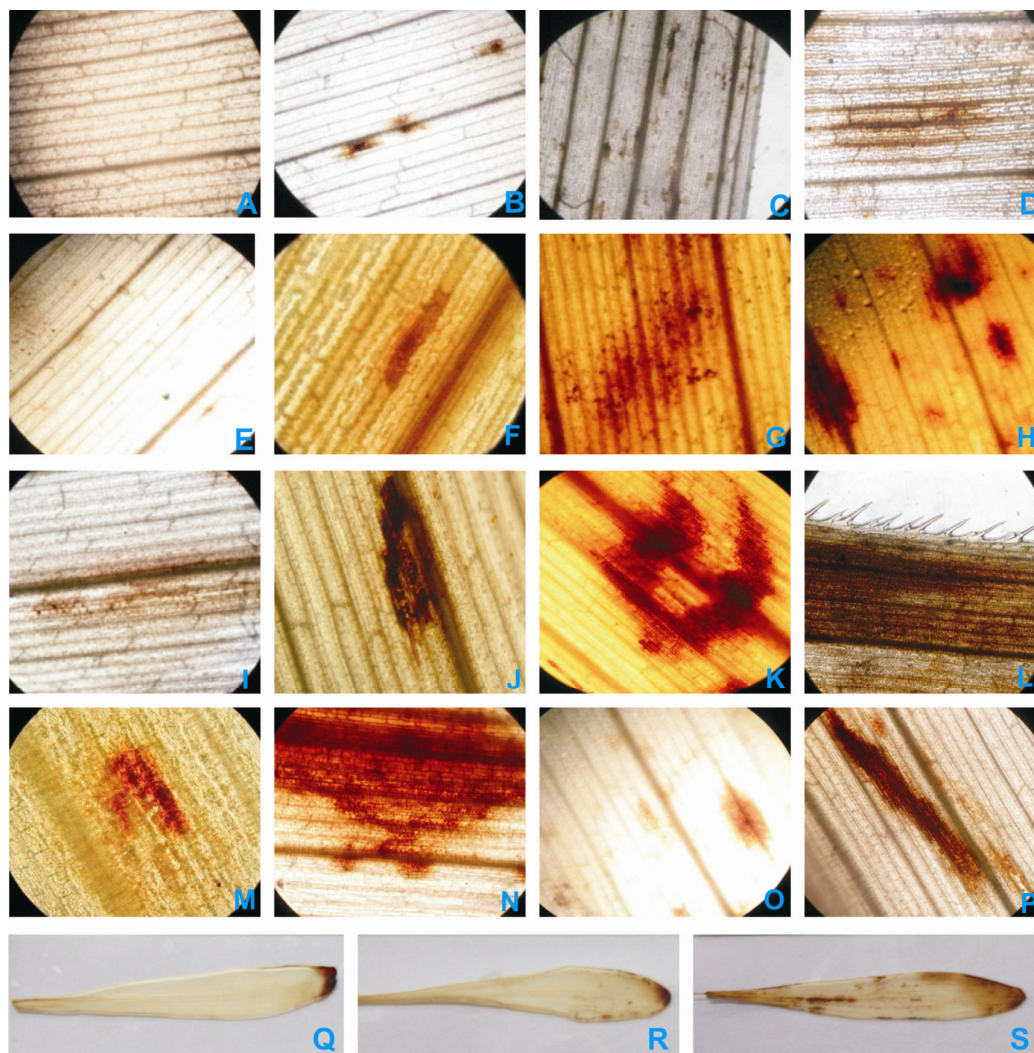


Fig 28B: Hydrogen peroxide localization by staining the leaves of maize cultivars subjected to drought stress with 3,3'Diaminobenzidine (DAB) and observing it in compound microscope (magnification 10X)

A,B,C,D: 0,3,5,7 day of drought stress *in vivo* in KS 244 respectively **E,F,G,H:** 0,3,5,7 day of drought stress *in vivo* in KS 244+ respectively **I,J,K,L,M,N,O,P:** 7-day drought stress *in vitro* in BN 101, 30V92, Swarna, Dhanya, KS 244, BN 1133, KS 244+ and 30V92 **Q,R,S:** 0-day, 3-day, 7-day leaf of BN 1133 during drought stress *in vitro*.

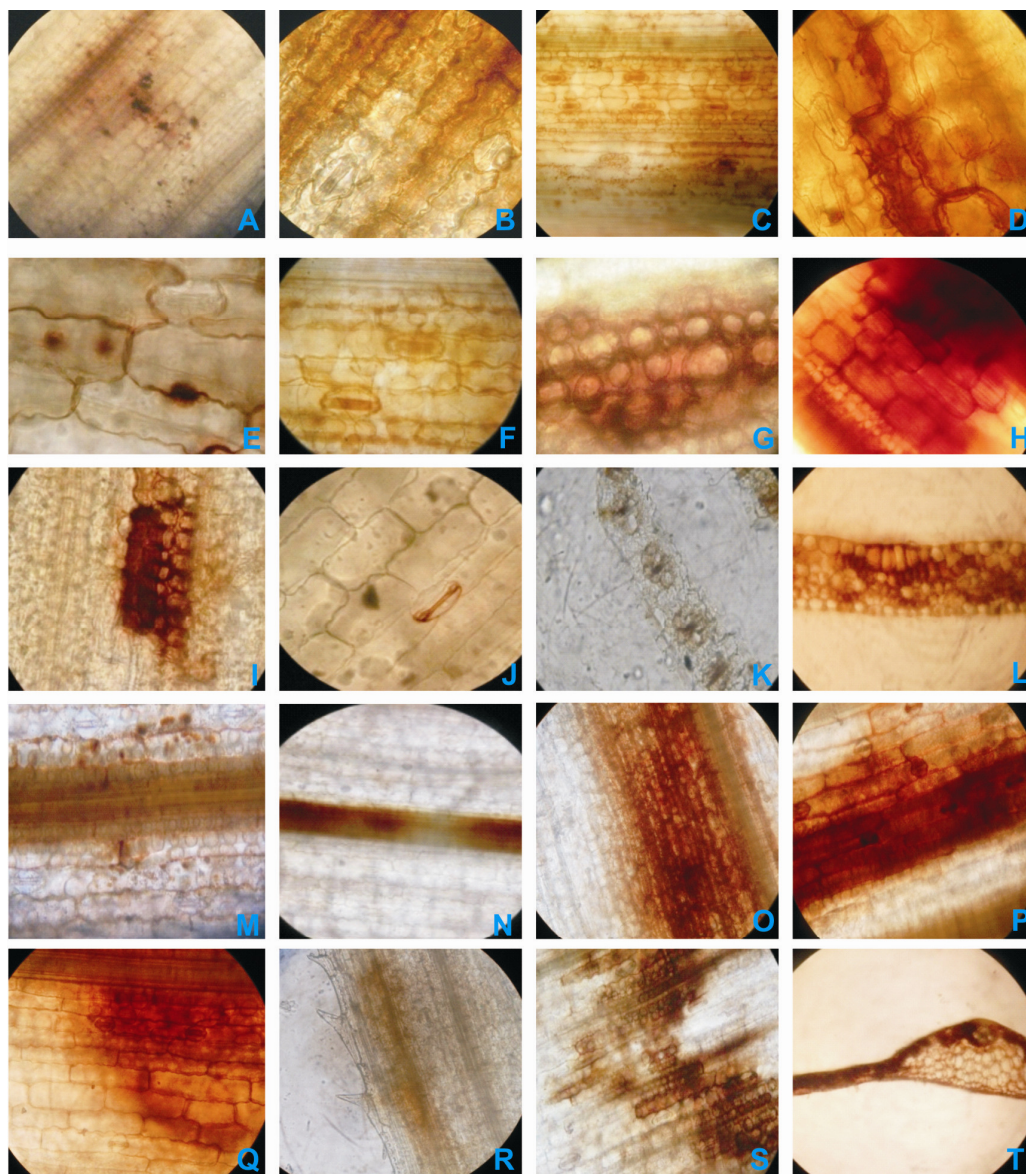


Fig 28C: Hydrogen peroxide localization by staining the leaves of seven cultivars of maize subjected to drought stress *in vivo* with 3,3'Diaminobenzidine (DAB) and observing it in compound microscope (40X)

A,B,C,D: BN 1133 leaf subjected to 7-day drought stress **E,F,G,H:** 30V92 leaf subjected to 7-day of drought stress **I, J:** BN 101 leaf subjected to 7-day drought stress **K,L:** T.S of leaf of Swarna subjected to 7-day drought stress **M,N,O,P:** Mid rib of BN 1133, BN 101, Dhanya and Swarna subjected to 7-day of drought **Q, R, S:** Leaf of Swarna subjected to 7-day drought stress **T:** T.S of Dhanya midrib and leaf respectively subjected to 7-day of drought

4.13. Effect of water stress on protein in different cultivars of maize subjected to water stress

4.13.1 Protein content

Protein content in the leaves and root of seven cultivars of maize showed various trends during water stress. In case of KS 244, KS 244+, BN 101 and 30V92 there was an increase in the protein content during both *in vitro* (Table 27) and *in vivo* (Table 28) drought stress and also in flooding stress (Table 29) in both the leaves and the roots of the test plant. But in the other three cultivars – BN 1133, Dhanya and Swarna there was a significant decline in the protein content after the 3rd day of drought stress, and the results were similar in case of both the leaves and the roots. The protein content was seen to be more in the leaves than in the roots.

4.13.2. Protein profile

Protein profile was analyzed by SDS-PAGE from the leaves of different cultivars of maize plant. Many protein bands were expressed in the maize cultivars during water stress *in vivo*. There was also disappearance of many protein bands in the cultivars when compared to 0-day of stress. In Swarna cultivar new band of 18 kDa was seen to be expressed on the 5th and 7th day of drought stress (Fig 29). In KS 244 on the 5th day of drought stress protein bands of 28, 21 and 18 kDa were expressed on the 5th day of drought stress, and on 3rd and 5th day of drought stress and 3rd, 5th and 7th day of flooding stress protein band of 28 kDa was seen to express (Fig 30). In Dhanya cultivar protein band of 24 kDa was expressed in 3rd, 5th and 7th day of drought stress, and on 5th and 7th day of stress another protein band of 31 kDa was also expressed (Fig 31). In KS 244+ cultivar a new protein band of 18 kDa was expressed on the 5th day of drought stress and 42 kDa on 7th day of flooding stress (Fig 32). In BN 1133 cultivar three new protein bands - 74 kDa, 48 kDa and 18 kDa were expressed during drought stress *in vivo* (Fig 33) and in BN 101, protein band of 18 kDa was expressed in 3rd, 5th and 7th day of drought stress. On 5th and 7th day of drought stress two more new protein bands of 74 and 35 kDa were also expressed (Fig 34).

Table 27: Protein content from the leaves and root of seven maize cultivars during drought stress *in vitro*

CULTIVARS	Protein content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	32.89± 2.222 ^a	34.67± 0.770 ^a	38.67± 0.770 ^a	44.44± 1.778 ^b	6.67± 0.770 ^a	12.44± 0.444 ^b	17.78± 0.444 ^b	26.67± 0.770 ^b
KS 244+	29.33± 0.770 ^a	32.89± 0.444 ^b	37.78± 0.444 ^b	48.44± 1.176 ^b	80.00± 0.770 ^a	14.67± 1.333 ^b	19.11± 0.444 ^b	26.22± 0.444 ^b
BN 101	21.78± 1.176 ^a	33.33± 0.770 ^b	39.11± 1.176 ^b	48.00± 0.770 ^b	7.56± 1.176 ^a	15.56± 1.176 ^b	20.44± 0.444 ^b	24.44± 0.444 ^b
30V92	24.00± 0.770 ^a	29.78± 1.176 ^b	33.78± 0.444 ^b	38.67± 0.770 ^b	8.89± 0.444 ^a	11.11± 0.444 ^b	15.56± 1.176 ^b	18.67± 0.770 ^b
BN 1133	28.44± 0.444 ^a	32.44± 0.889 ^b	31.55± 1.780 ^a	30.22± 1.600 ^a	7.56± 0.889 ^a	9.78± 1.602 ^a	12.44± 0.444 ^b	10.22± 0.890 ^a
DHANYA	25.78± 0.444 ^a	32.89± 0.444 ^b	31.99± 0.770 ^b	27.11± 0.889 ^a	7.11± 0.444 ^a	12.00± 0.770 ^b	11.11± 0.890 ^b	10.22± 0.890 ^a
SWARNA	28.00± 0.770 ^a	34.22± 0.444 ^b	32.89± 2.350 ^a	30.67± 2.670 ^a	7.11± 1.176 ^a	13.33± 1.333 ^b	12.44± 0.444 ^b	11.99± 2.040 ^a

LEAF: CD- Treatments: 5.13; Cultivars: 6.79 ROOT: CD- Treatments: 3.85; Cultivars: 5.09
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 27A: ANOVA of data presented in Table 27 (Leaf)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	456.2694	3	152.0898	7.275065	0.002135	3.159908
Columns	267.8913	6	44.64855	2.135719	0.099186	2.661305
Error	376.3013	18	20.90563			
Total	1100.462	27				

Table 27B: ANOVA of data presented in Table 27 (Root)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	469.2273	3	156.4091	13.29523	8.13E-05	3.159908
Columns	233.4425	6	38.90708	3.307214	0.022544	2.661305
Error	211.7575	18	11.76431			
Total	914.4273	27				

Table 28: Protein content in from the leaves and root of maize cultivars during drought stress *in vivo*

CULTIVARS	Protein content (mg g ⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	23.56± 0.444 ^a	33.78± 0.444 ^b	34.67± 1.540 ^b	44.89± 1.602 ^b	10.22± 0.444 ^a	14.67± 0.770 ^a	17.33± 0.770 ^b	21.78± 0.444 ^b
KS 244+	27.56± 0.444 ^a	36.44± 0.889 ^b	40.44± 0.444 ^b	48.89± 0.444 ^b	12.89± 0.889 ^a	14.67± 0.770 ^a	17.33± 0.444 ^b	20.89± 0.444 ^b
BN 101	25.78± 1.176 ^a	35.11± 0.444 ^b	36.44± 0.889 ^b	42.22± 0.444 ^b	9.78± 0.444 ^a	15.56± 0.444 ^a	19.56± 0.444 ^b	21.78± 0.444 ^b
30V92	19.99± 0.444 ^a	21.33± 0.770 ^a	35.11± 0.444 ^b	42.67± 0.770 ^b	11.56± 0.444 ^a	12.89± 0.444 ^a	17.33± 0.770 ^b	20.44± 0.444 ^b
BN 1133	24.44± 1.176 ^a	34.67± 1.540 ^b	31.56± 0.444 ^a	27.11± 0.444 ^b	8.44± 0.444 ^a	12.44± 0.444 ^a	10.22± 0.444 ^b	9.78± 0.444 ^b
DHANYA	23.11± 0.889 ^a	27.11± 1.180 ^a	26.22± 0.444 ^a	25.33± 1.330 ^a	11.11± 0.444 ^a	14.67± 0.770 ^b	12.44± 0.444 ^a	11.56± 0.889 ^a
SWARNA	23.56± 0.444 ^a	32.44± 0.889 ^b	29.78± 1.780 ^b	25.33± 0.770 ^b	10.67± 0.770 ^a	14.67± 0.770 ^a	14.22± 0.889 ^a	12.89± 0.444 ^a

LEAF: CD- Treatment: 5.83; Cultivars: 7.71 ROOT: CD- Treatment: 2.79; Cultivars: 3.70
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 28A: ANOVA of data presented in Table 28 (Leaf)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	618.2287	3	206.0762	7.653595	0.001674	3.159908
Columns	496.1334	6	82.6889	3.071035	0.029984	2.661305
Error	484.6575	18	26.92542			
Total	1599.02	27				

Table 29: Protein content in the leaves and root of maize during flooding stress

CULTIVARS	Protein content (mg g⁻¹ tissue FW)							
	Leaf				Root			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	21.78± 2.222 ^a	35.56± 1.937 ^b	46.22± 0.889 ^b	48.89± 0.889 ^b	12.89± 0.444 ^a	17.33± 1.333 ^b	20.44± 0.444 ^b	24.89± 0.444 ^b
KS 244+	28.44± 0.444 ^a	38.67± 0.770 ^b	44.89± 1.602 ^b	52.44± 0.444 ^b	13.78± 0.444 ^a	17.78± 0.889 ^b	18.67± 0.770 ^b	21.33± 0.770 ^b
BN 101	27.11± 0.889 ^a	36.44± 1.602 ^b	50.22± 3.950 ^b	42.67± 3.528 ^b	12.00± 0.770 ^a	17.33± 0.770 ^b	23.56± 0.444 ^b	24.89± 0.444 ^b
30V92	19.11± 0.444 ^b	26.67± 1.540 ^b	46.67± 0.770 ^b	30.67± 0.770 ^b	10.21± 0.444 ^a	15.54± 1.175 ^b	16.87± 0.888 ^a	12.00± 1.333 ^a
BN 1133	24.44± 1.176 ^a	28.00± 1.540 ^a	26.67± 0.770 ^a	24.89± 0.444 ^a	9.78± 1.602 ^a	15.56± 1.176 ^b	14.67± 0.770 ^a	10.22± 0.889 ^a
DHANYA	23.11± 0.889 ^a	32.89± 0.444 ^b	30.22± 1.176 ^b	28.89± 0.889 ^b	9.33± 1.333 ^a	14.67± 0.770 ^b	13.78± 0.444 ^a	12.00± 0.770 ^a
SWARNA	23.56± 0.444 ^a	29.78± 2.475 ^a	28.89± 0.444 ^b	21.78± 1.602 ^a	11.11± 0.889 ^a	16.00± 1.333 ^a	13.78± 0.444 ^a	12.89± 0.444 ^a

LEAF: CD- Treatment: 6.87; Cultivars: 9.08 ROOT: CD- Treatment: 3.01; Cultivars: 3.99

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 28B: ANOVA of data presented in Table 28 (Root)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	155.0182	3	51.67272	8.350977	0.001086	3.159908
Columns	146.46	6	24.40999	3.944969	0.010804	2.661305
Error	111.3773	18	6.187626			
Total	412.8554	27				

Table 29A: ANOVA of data presented in Table 29 (Leaf)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	889.8499	3	296.6166	7.935175	0.001402	3.159908
Columns	999.2905	6	166.5484	4.455552	0.006209	2.661305
Error	672.8395	18	37.37997			
Total	2561.98	27				

Table 29B: ANOVA of data presented in Table 29 (Root)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	166.7598	3	55.58658	7.696583	0.001629	3.159908
Columns	233.4589	6	38.90981	5.387498	0.002428	2.661305
Error	130.0004	18	7.222242			
Total	530.219	27				

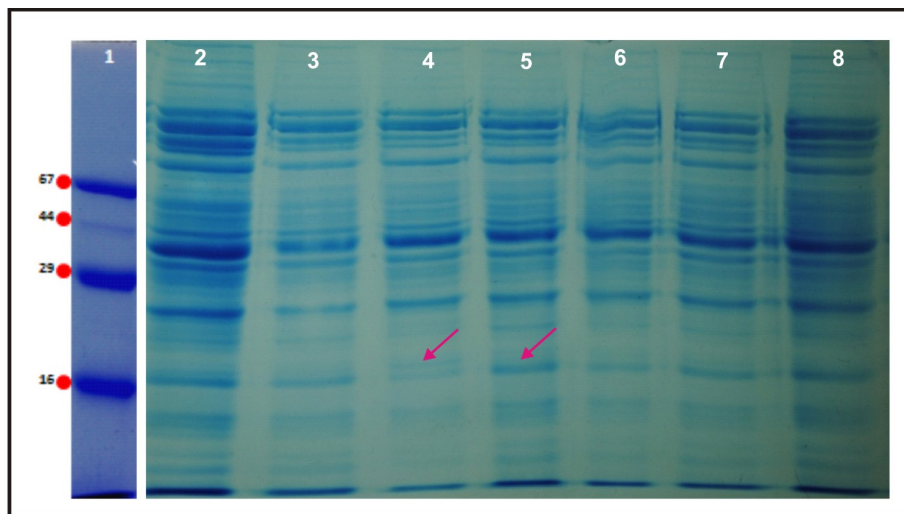


Fig 29: SDS-PAGE analysis of protein leaves in Swarna cultivar during drought and flooding stress *in vivo* condition. **Lane 1:** Molecular marker, **Lane 2:** 0-day, **Lane 3:** 3-day drought, **Lane 4:** 5-day drought, **Lane 5:** 7-day drought, **Lane 6:** 3-day flooding, **Lane 7:** 5-day flooding, **Lane 8:** 7-day flooding

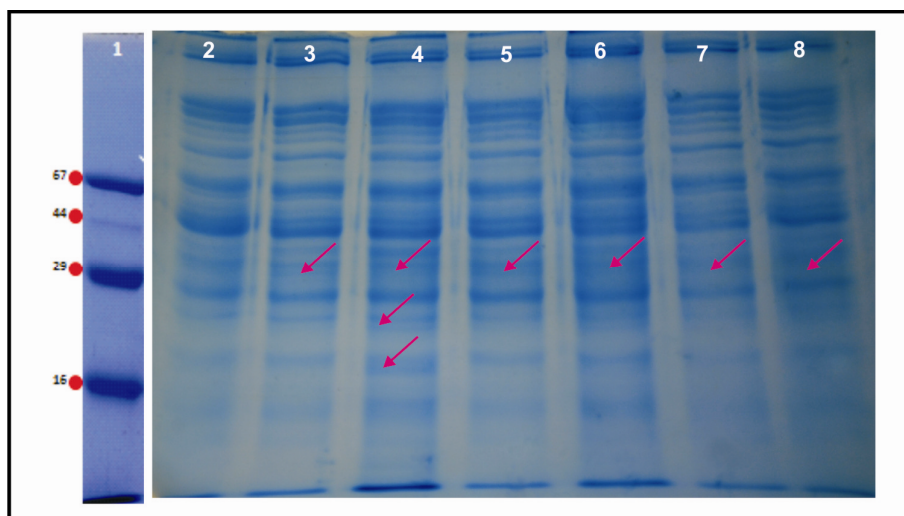


Fig 30: SDS-PAGE analysis of protein leaves in KS 244 cultivar during water stress *in vivo* condition. **Lane 1:** Molecular marker, **Lane 2:** 0-day, **Lane 3:** 3-day drought, **Lane 4:** 5-day drought, **Lane 5:** 7-day drought, **Lane 6:** 3-day flooding, **Lane 7:** 5-day flooding, **Lane 8:** 7-day flooding

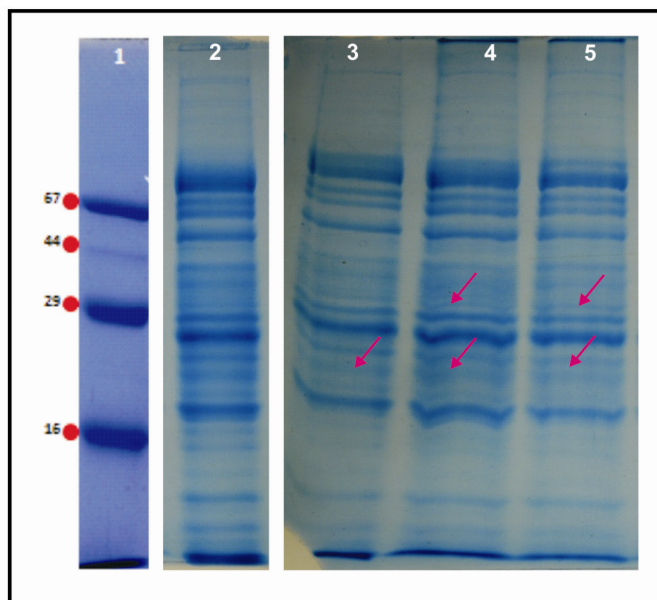


Fig 31: SDS-PAGE analysis of protein leaves in Dhanya cultivar during drought *in vivo*. **Lane 1:** Molecular marker, **Lane 2:** 0-day, **Lane 3:** 3-day, **Lane 4:** 5-day, **Lane 5:** 7-day

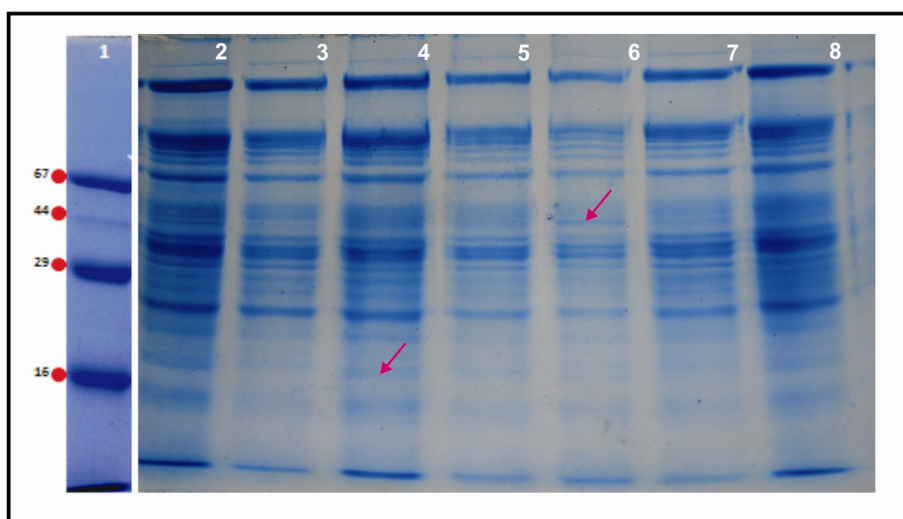


Fig 32: SDS-PAGE analysis of protein leaves in KS 244+ cultivar during water stress. **Lane 1:** Molecular marker, **Lane 2:** 0-day, **Lane 3:** 3-day drought, **Lane 4:** 5-day drought, **Lane 5:** 7-day drought, **Lane 6:** 7-day flooding, **Lane 7:** 5-day flooding, **Lane 8:** 3-day flooding

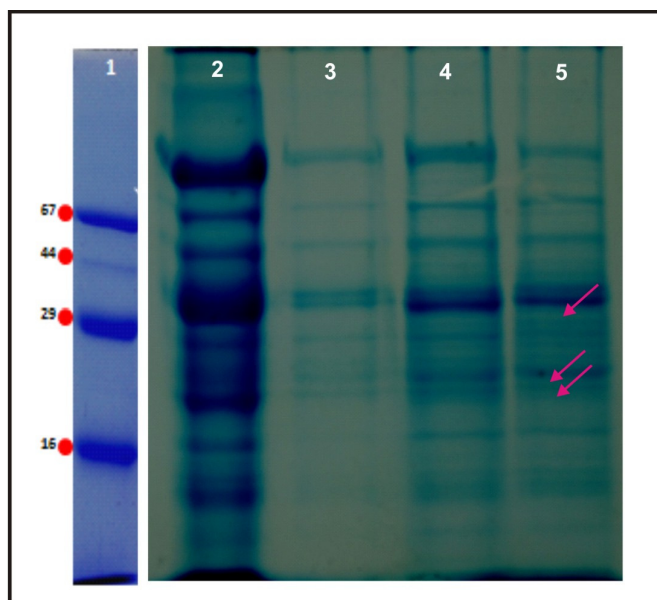


Fig 33: SDS-PAGE analysis of protein leaves in BN 1133 cultivar during drought stress *in vivo*. **Lane 1:** Molecular marker, **Lane 2:** 0-day, **Lane 3:** 3-day, **Lane 4:** 5-day, **Lane 5:** 7-day

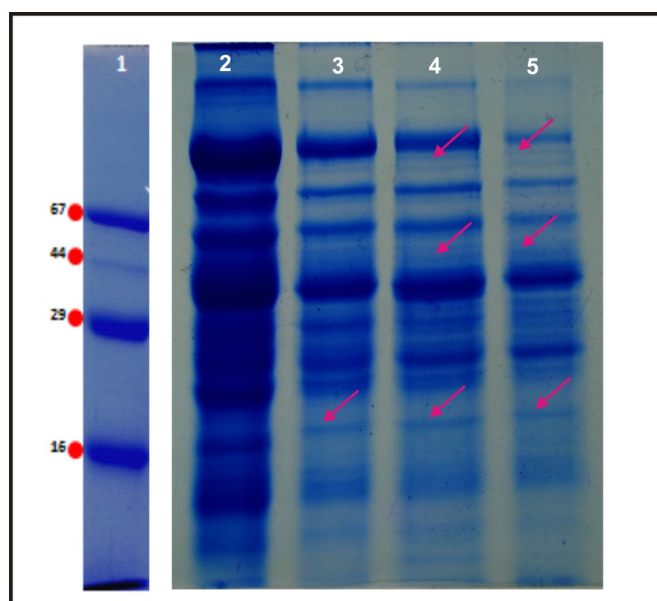


Fig 34: SDS-PAGE analysis of protein leaves in BN 101 cultivar during drought stress *in vivo*. **Lane 1:** Molecular marker, **Lane 2:** 0-day, **Lane 3:** 3-day, **Lane 4:** 5-day, **Lane 5:** 7-day

Table 30: Protein bands from the leaves of three cultivars of maize during drought and flooding stress *in vivo* as analyzed by SDS- PAGE

Cultivar	Treatment	Lane Number	No. of bands	Molecular mass of Protein bands(kDa)
SWARNA	0-day drought	1	21	85,83,82,81,78,76,68,67,52,44,36,34,29,25, 20,16,13,10,9,6,3
	3-day drought	2	20	85,83,82,81,78,76,68,67,52,44,36,34,29,25, 20,16,13,10,6,3
	5-day drought	3	21	85,83,82,81,78,76,68,67,52,44,36,34,29,25 20, 18 ,16,13,10,6,3
	7-day drought	4	21	85,83,82,81,78,76,68,67,52,44,36,34,29,25, 20, 18 ,16,13,10,6,3
	3-day flooding	5	20	85,83,82,81,78,76,68,67,52,44,36,34,29,25, 20,16,13,10,6,3
	5-day flooding	6	20	85,83,82,81,78,76,68,67,52,44,36,34,29,25, 20,16,13,10,6,3
	7-day flooding	7	20	85,83,82,81,78,76,68,67,52,44,36,34,29,25, 20,16,13,10,6,3
KS 244	0-day drought	1	20	103,101,89,85,80,77,73,70,67,63,61,44,40, 35,29,27,25,19,14,9
	3-day drought	2	21	103,101,89,85,80,77,73,70,67,63,61,44,40, 35, 28 ,29,27,25,19,14,9
	5-day drought	3	23	103,101,89,85,80,77,73,70,67,63,61,44,40, 35, 28 ,29,27,25, 21 ,19, 18 ,14,9
	7-day drought	4	21	103,101,89,85,80,77,73,70,67,63,61,44,40, 35, 28 ,29,27,25,19,14,9
	3-day flooding	5	22	103,101,89,85,80,77,73,70,67,63,61,44,40, 35, 28 ,29,27,25,19,18,14,9
	5-day flooding	6	21	103,101,89,85,80,77,73,70,67,63,61,44,40, 35, 28 ,29,27,25,19,14,9
	7-day flooding	7	21	103,101,89,85,80,77,73,70,67,63,61,44,40, 35, 28 ,29,27,25,19,14,9
DHANYA	0-day drought	1	26	93,88,78,75,72,69,67,54,50,44,39,29,27,25, 23,21,20,18,17,16,15,11,9,7,5,4
	3-day drought	2	23	93,88,78,75,72,69,54,44,39,29,27,25, 24 ,23, 21,20,18,17,15,11,9,7,4
	5-day drought	3	24	93,88,78,75,72,67,69,54,50,44,39, 31 ,29,27, 24 ,23,21,18,15,9,7,4
	7-day drought	4	23	93,88,78,75,72,67,69,54,44,39, 31 ,29,27,25, 24 ,23,21,18,15,9,7,4

Table 31: Protein bands from the leaves of three cultivars of maize during drought and flooding stress *in vivo* as analyzed by SDS- PAGE

Cultivar	Treatment	Lane Number	No. of bands	Molecular mass of Protein bands(kDa)
KS 244+	0-day drought	1	20	90,81,77,74,70,68,67,49,44,38,32,29,27, 25,22,20,19,16,14,12
	3-day drought	2	20	90,81,77,74,70,68,67,49,44,38,32,29,27, 25,22,20,19,16,14,12
	5-day drought	3	21	90,81,77,74,70,68,67,49,44,38,32,29,27, 25,22,20,19, 18 ,16,14,12
	7-day drought	4	20	90,81,77,74,70,68,67,49,44,38,32,29,27, 25,22,20,19, ,16,14,12
	7-day flooding	5	21	90,81,77,74,70,68,67,49,44, 42 ,38,32,29, 27,25,22,20,19,16,14,12
	5-day flooding	6	20	90,81,77,74,70,68,67,49,44,38,32,29,27, 25,22,20,19,16,14,12
	3-day flooding	7	20	90,81,77,74,70,68,67,49,44,38,32,29,27, 25,22,20,19,16,14,12
BN 1133	0-day drought	1	13	72,64,56,37,35,27,21,19,16,14,12,11,5
	3-day drought	2	9	72,64,56,37,35,27,21,19,5
	5-day drought	3	13	72,64,56,37,35,27,21,19,16,14,12,11,5
	7-day drought	4	16	72,64,56,37,35, 30 ,27, 25,23 ,21,19,16,14, 12,11,5
BN 101	0-day drought	1	20	93,89,77,76,7,69,67,60,55,44,37,32,29, 27,25,23,20,16,10,7
	3-day drought	2	21	93,89,77,76,72,69,67,60,55,44,37,32,29, 27,25,23,20, 18 ,16,10,7
	5-day drought	3	23	93,89,77,76, 74 ,72,69,67,60,55,48,44,37, 32,29,27,25,23,20, 18 ,16,10,7
	7-day drought	4	23	93,89,77,76, 74 ,72,69,67,60,55,48,44,37, 32,29,27,25,23,20, 18 ,16,10,7

4.14. Changes in phenolics during drought stress

4.14.1. Phenol content

The phenol content (Table 32, Table 33 and Table 34) from the leaves of seven cultivars of maize during all three sets of water stress decreased during the period of stress. Maximum decrease in the phenolics was seen in case of Dhanya, Swarna, 30V92 and BN 1133, where the decrease was almost twice of that of BN 101, KS 244 and KS 244+. ANOVA of data of phenol content showed a very high significant difference among the cultivars in case of phenolic content during water stress.

4.14.2. HPLC profiles of phenols

HPLC profiles of total phenol and phenolic acids were performed from the leaves of two cultivars of maize-KS 244 and Dhanya during drought and flooding stress in pot culture. In the HPLC of total phenol an unidentified peak having a retention time of 9.70 mins was very much prominent in both cultivars and in both drought and flooding stress. A reduction in the peak in the stressed plant in both the cultivars was observed when compared to the control (Fig 35 and Fig 36). Certain peaks of total phenol have been seen to disappear during both drought and flooding stress in both KS 244 and Dhanya cultivar. In the HPLC analysis of phenolic acid, the control plant of KS 244, showed the following peaks - gallic acid, caffeic acid, vanillic acid and cinnamic acid, but during 3rd, 5th and 7th day of drought stress, HPLC analysis showed the disappearance of all the peaks except for vanillic acid. During flooding stress in the same cultivar the peaks corresponding to caffeic acid and gallic acid disappeared since the 3rd day of flooding stress, and on the 7th day of stress only the peak corresponding to vanillic acid was visible. In the control plant of Dhanya cultivar two peaks-ferulic acid and cinnamic acid were detected, but on the 5th and 7th day of drought stress, the peak corresponding to cinnamic acid had disappeared and only peak corresponding to ferulic acid was detected. Similar results were obtained from plants subjected to flooding stress in the same Dhanya. Therefore overall results of HPLC analysis of total phenols and phenolic acid of the two cultivars-KS 244 and Dhanya showed the reduction and even disappearance of most of the peaks during the period of water stress (Table 35 and 36), especially in case of Dhanya.

Table 32: Phenolic content of leaves of maize cultivars during drought stress
in vitro

CULTIVARS	Phenol content (mg g ⁻¹ tissue FW)							
	Total Phenol				Ortho-dihydroxy phenol			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	4.07± 0.134 ^a	3.85± 0.037 ^a	3.07± 0.098 ^b	2.89± 0.128 ^b	3.13± 0.240 ^a	2.73± 0.067 ^a	2.33± 0.176 ^a	1.67± 0.067 ^b
KS 244+	3.89± 0.231 ^a	3.85± 0.148 ^a	2.89± 0.111 ^b	2.78± 0.064 ^b	3.00± 0.115 ^a	2.20± 0.115 ^a	1.33± 0.570 ^b	1.13± 0.176 ^b
BN 101	3.85± 0.037 ^a	3.04± 0.196 ^b	2.44± 0.064 ^b	2.04± 0.037 ^b	3.53± 0.067 ^a	2.87± 0.133 ^a	2.27± 0.176 ^b	1.13± 0.133 ^b
30V92	2.52± 0.098 ^a	2.33± 0.064 ^a	1.70± 0.037 ^b	1.74± 0.037 ^b	2.80± 0.115 ^a	1.87± 0.067 ^a	1.00± 0.115 ^b	0.67± 0.067 ^b
BN 1133	2.89± 0.128 ^a	1.85± 0.098 ^b	1.67± 0.064 ^b	1.37± 0.037 ^b	3.33± 0.176 ^a	2.33± 0.067 ^a	1.4± 0.115 ^b	0.67± 0.067 ^b
DHANYA	2.59± 0.037 ^a	1.56± 0.064 ^b	1.30± 0.037 ^b	0.81± 0.037 ^b	2.47± 0.067 ^a	2.07± 0.067 ^a	0.87± 0.067 ^b	0.27± 0.067 ^b
SWARNA	3.81± 0.09 ^a	2.44± 0.128 ^b	1.85± 0.037 ^b	1.26± 0.134 ^b	2.47± 0.176 ^a	1.67± 0.067 ^a	0.93± 0.067 ^b	0.60± 0.115 ^b

Total phenol: CD- Treatments: 0.32; Cultivars- 0.42

Ortho-dihydroxy phenol: CD- Treatments: 0.26; Cultivars- 0.34

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

Table 32A: ANOVA of data presented in Table 32 (Total phenol)

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	9.636537	3	3.212179	40.78341	3.11E-08	3.159908
Columns	12.70495	6	2.117492	26.88473	4.71E-08	2.661305
Error	1.417714	18	0.078762			
Total	23.7592	27				

Table 32B: ANOVA of data presented in Table 32 (Ortho-dihydroxy phenol)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	17.50143	3	5.83381	109.4201	9.54E-12	3.159908
Columns	4.762222	6	0.793704	14.88687	4.17E-06	2.661305
Error	0.959683	18	0.053316			
Total	23.22333	27				

Table 33: Phenol contents of leaves of maize cultivars during drought stress *in vivo*

CULTIVARS	Phenol content (mg g⁻¹ tissue FW)							
	Total phenol				Ortho-dihydroxy phenol			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	3.78± 0.128 ^a	2.67± 0.064 ^b	2.59± 0.074 ^b	2.04± 0.037 ^b	2.13± 0.133 ^a	1.27± 0.527 ^b	1.47± 0.067 ^b	0.93± 0.067 ^b
KS 244+	3.30± 0.037 ^a	2.81± 0.98 ^b	1.96± 0.037 ^b	1.59± 0.037 ^b	2.07± 0.067 ^a	1.93± 0.067 ^a	1.47± 0.067 ^b	1.06± 0.176 ^b
BN 101	3.89± 0.257 ^a	3.81± 0.134 ^b	2.59± 0.185 ^b	2.07± 0.037 ^b	2.27± 0.067 ^a	2.06± 0.176 ^a	1.60± 0.115 ^b	1.33± 0.067 ^b
30V92	3.81± 0.037 ^a	2.70± 0.161 ^b	1.81± 0.134 ^b	1.41± 0.161 ^b	2.67± 0.133 ^a	1.67± 0.133 ^b	1.53± 0.067 ^b	0.87± 0.067 ^b
BN 1133	3.30± 0.037 ^a	2.85± 0.074 ^b	1.63± 0.037 ^b	1.15± 0.037 ^b	2.53± 0.133 ^a	1.67± 0.133 ^b	0.93± 0.067 ^b	0.73± 0.067 ^b
DHANYA	3.96± 0.133 ^a	2.93± 0.097 ^b	1.48± 0.074 ^b	0.93± 0.037 ^b	2.40± 0.200 ^a	1.93± 0.067 ^b	0.93± 0.067 ^b	0.53± 0.067 ^b
SWARNA	3.59± 0.098 ^a	2.63± 0.196 ^b	2.03± 0.074 ^b	1.56± 0.064 ^b	2.60± 0.200 ^a	1.87± 0.067 ^b	0.73± 0.067 ^b	0.47± 0.067 ^b

Total phenol: CD- Treatments: 0.38; Cultivars- 0.51

Ortho-dihydroxy phenol: CD- Treatments: 0.50; Cultivars- 0.67

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at $P \leq 0.05$ in Students 't' test within each cultivar.

Table 33A: ANOVA of data presented in Table 33 (Total phenol)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	20.44019	3	6.813398	58.83295	1.68E-09	3.159908
Columns	2.203841	6	0.367307	3.171654	0.026531	2.661305
Error	2.084566	18	0.115809			
Total	24.7286	27				

Table 34: Phenol content of leaves of maize cultivars during flooding stress

CULTIVARS	Phenol content (mg g⁻¹ tissue FW)							
	Total phenol				Ortho-dihydroxy phenol			
	0d	3d	5d	7d	0d	3d	5d	7d
KS 244	6.30± 0.259 ^a	5.48± 0.037 ^a	3.89± 0.064 ^b	3.15± 0.037 ^b	2.33± 0.133 ^a	1.33± 0.570 ^a	1.53± 0.067 ^b	1.33± 0.067 ^b
KS 244+	6.63± 0.243 ^a	6.30± 0.074 ^a	3.96± 0.037 ^b	2.70± 0.037 ^b	2.33± 0.067 ^a	1.33± 0.570 ^a	1.53± 0.067 ^b	1.27± 0.067 ^b
BN 101	6.81± 0.643 ^a	5.00± 0.257 ^a	3.85± 0.161 ^b	2.78± 0.192 ^b	2.07± 0.176 ^a	1.53± 0.067 ^a	1.19± 0.510 ^a	1.27± 0.133 ^b
30V92	5.78± 0.128 ^a	4.59± 0.098 ^b	3.67± 0.170 ^b	1.59± 0.074 ^b	2.27± 0.067 ^a	1.87± 0.067 ^b	0.93± 0.067 ^b	0.73± 0.067 ^b
BN 1133	4.04± 0.098 ^a	2.78± 0.231 ^b	2.74± 0.037 ^b	1.74± 0.074 ^b	1.73± 0.067 ^a	1.67± 0.067 ^a	0.8± 0.1155 ^b	0.53± 0.067 ^b
DHANYA	3.89± 0.111 ^a	3.07± 0.037 ^b	1.85± 0.098 ^b	1.04± 0.098 ^b	1.67± 0.133 ^a	0.93± 0.067 ^b	0.6± 0.115 ^b	0.33± 0.067 ^b
SWARNA	4.11± 0.280 ^a	2.89± 0.170 ^b	1.85± 0.098 ^b	1.22± 0.111 ^b	2.07± 0.067 ^a	1.67± 0.064 ^b	0.93± 0.133 ^b	0.53± 0.067 ^b

Total phenol: CD- Treatments: 0.51; Cultivars- 0.67

Ortho-dihydroxy phenol: CD- Treatments: 0.28 ; Cultivars- 0.37

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in students 't' test within each cultivar.

Table 33B: ANOVA of data presented in Table 32 (Ortho-dihydroxy phenol)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	9.631257	3	3.210419	15.98437	2.57E-05	3.159908
Columns	1.389254	6	0.231542	1.152827	0.373244	2.661305
Error	3.615254	18	0.200847			
Total	14.63577	27				

Table 34A: ANOVA of data presented in Table 34 (Total phenol)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	43.80354	3	14.60118	71.87268	3.25E-10	3.159908
Columns	28.03615	6	4.672692	23.0008	1.6E-07	2.661305
Error	3.656762	18	0.203153			
Total	75.49646	27				

Table 34B: ANOVA of data presented in Table 34 (Ortho-dihydroxy phenol)

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	5.927092	3	1.975697	31.72467	2.12E-07	3.159908
Columns	1.735863	6	0.289311	4.645592	0.005089	2.661305
Error	1.120975	18	0.062276			
Total	8.78393	27				

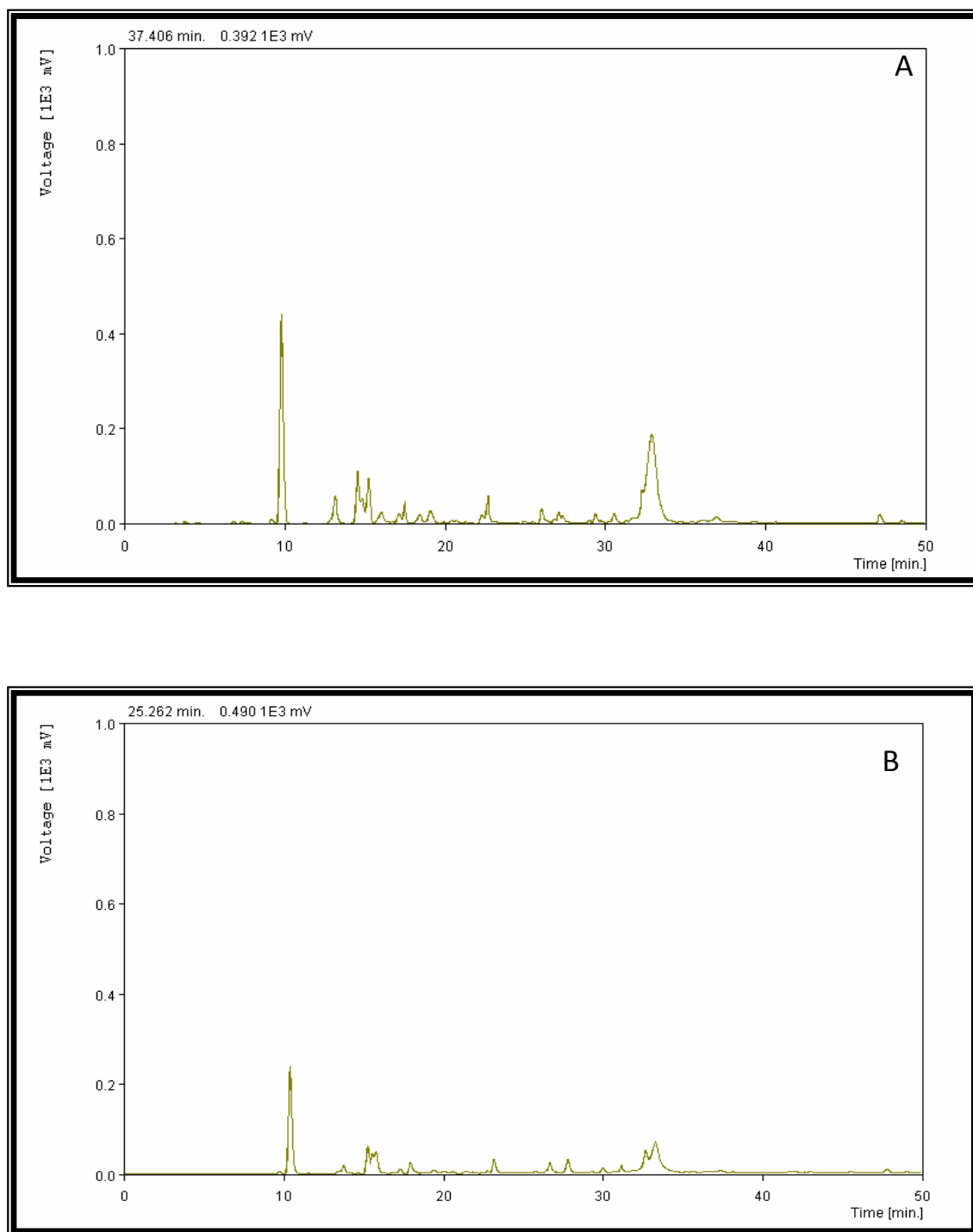


Fig 35A: HPLC analysis of total phenols from leaves of KS 244 cultivar during drought stress A: 0-day & B: 3-day

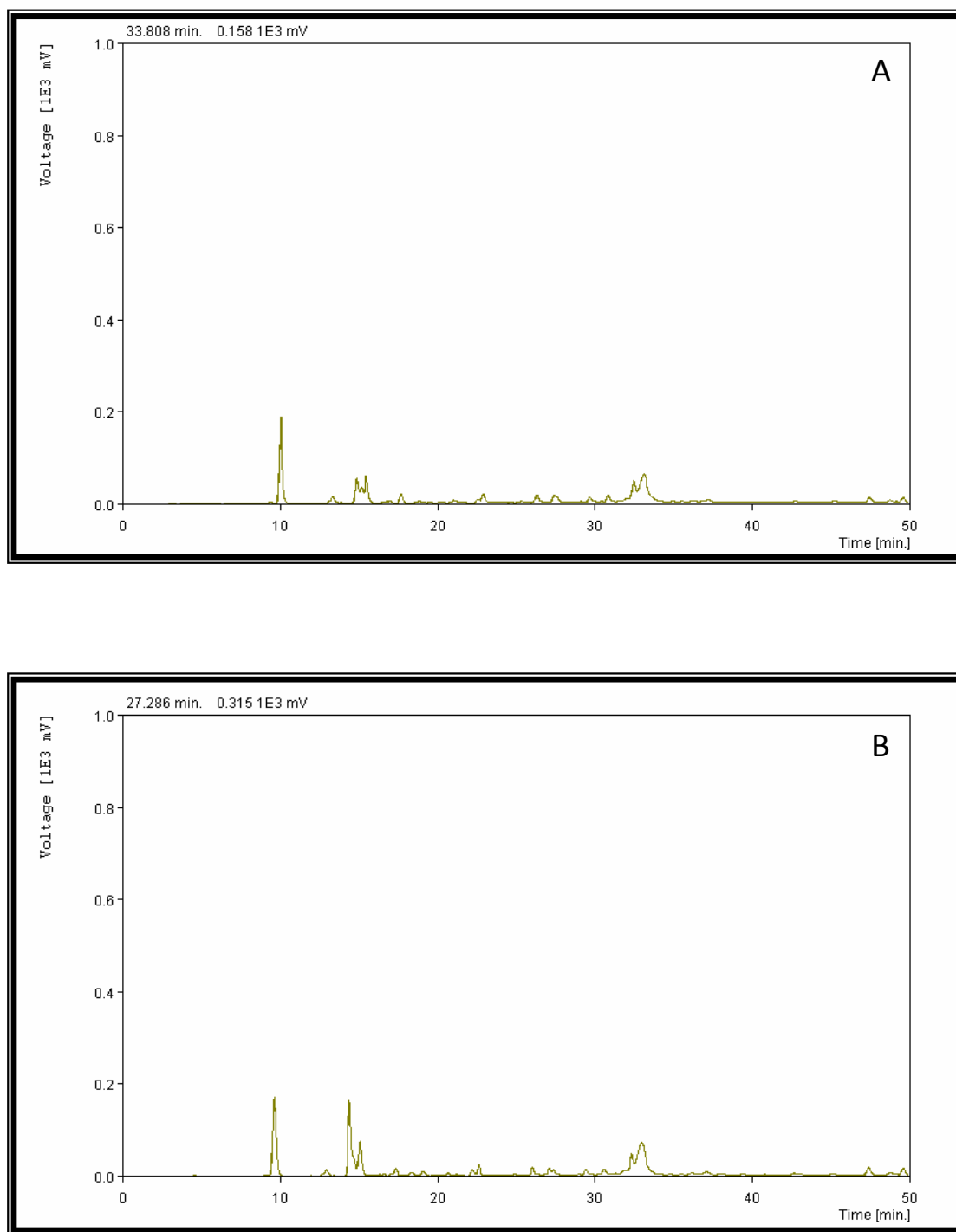


Fig 35B: HPLC analysis of total phenols from leaves of KS 244 cultivar during drought stress A: 5-day & B: 7-day

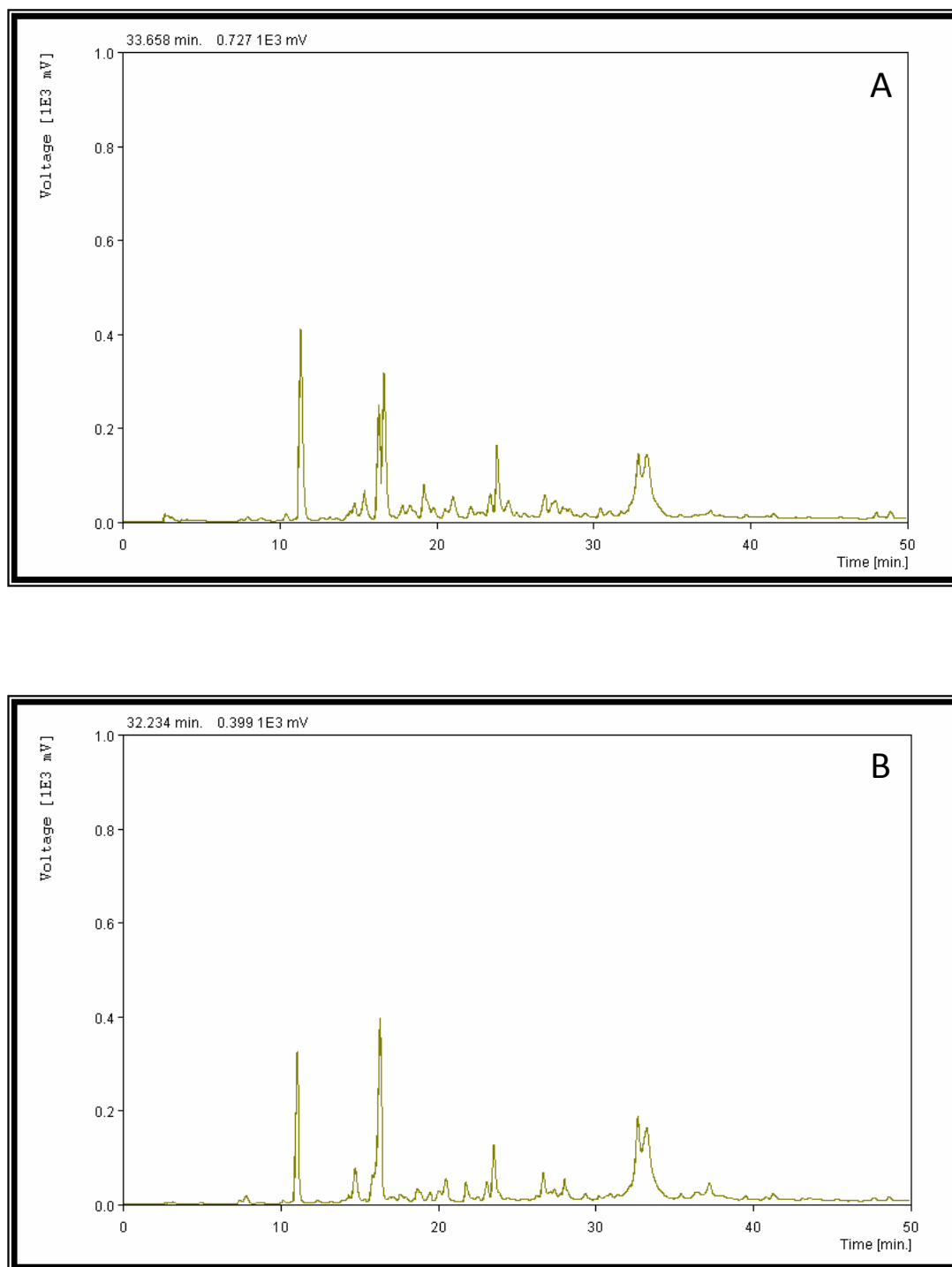


Fig 35C: HPLC analysis of total phenols from leaves of Dhanya cultivar during drought stress A: 0-day & B: 3-day

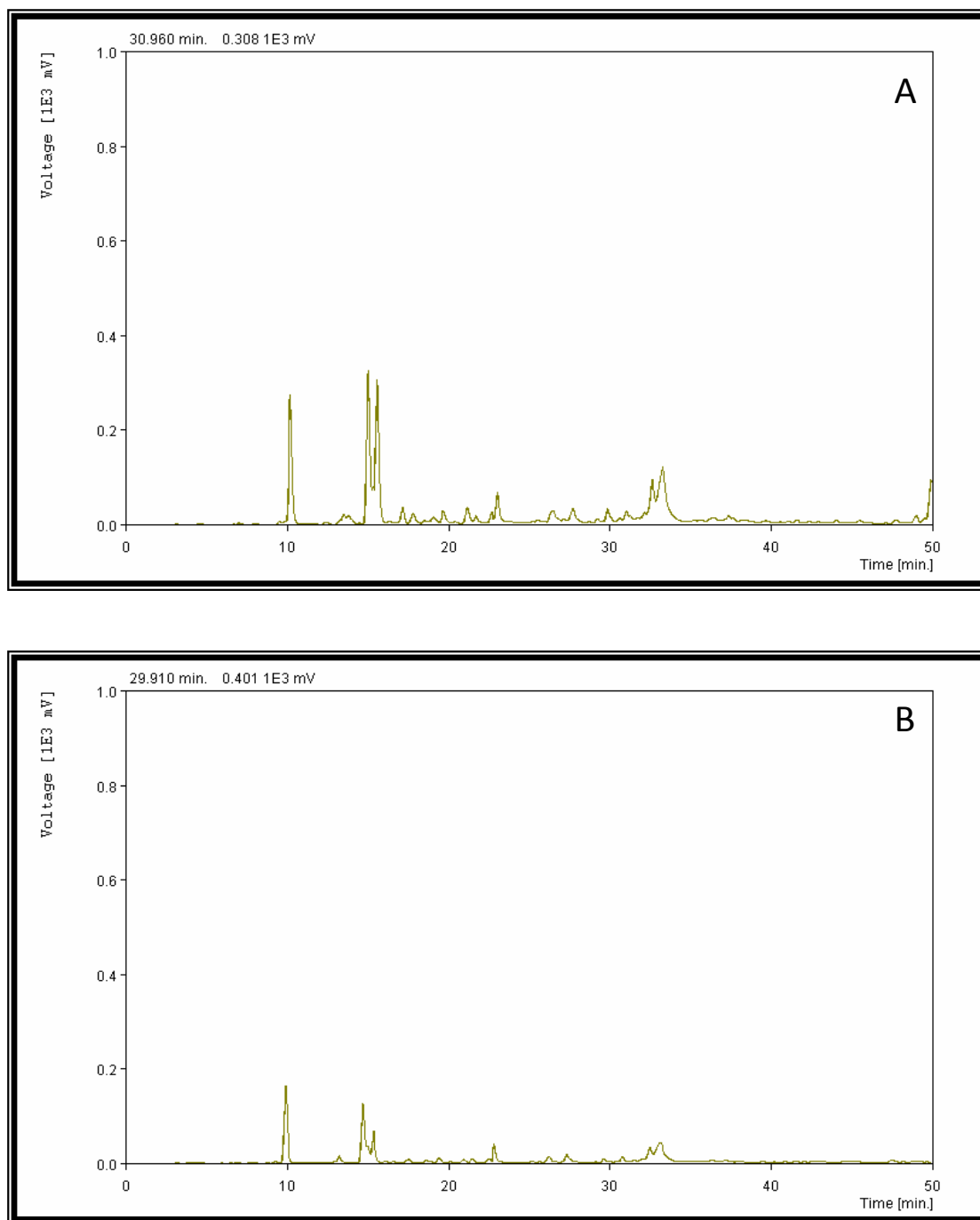


Fig 35D: HPLC analysis of total phenols from leaves of Dhanya cultivar during drought stress A: 5-day & B: 7-day

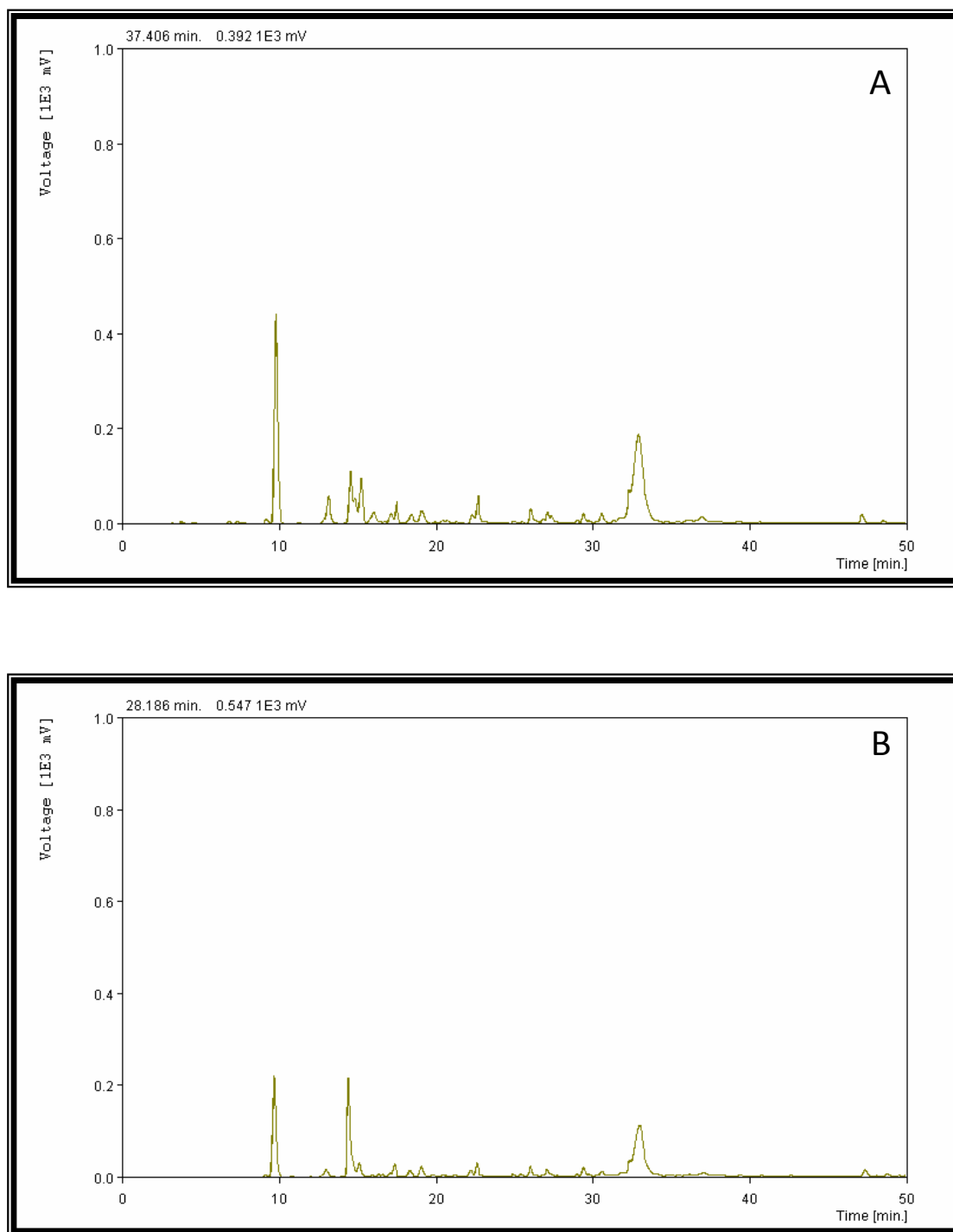


Fig 35E: HPLC analysis of total phenols from leaves of KS 244 cultivar during flooding stress A: 0-day & B: 3-day

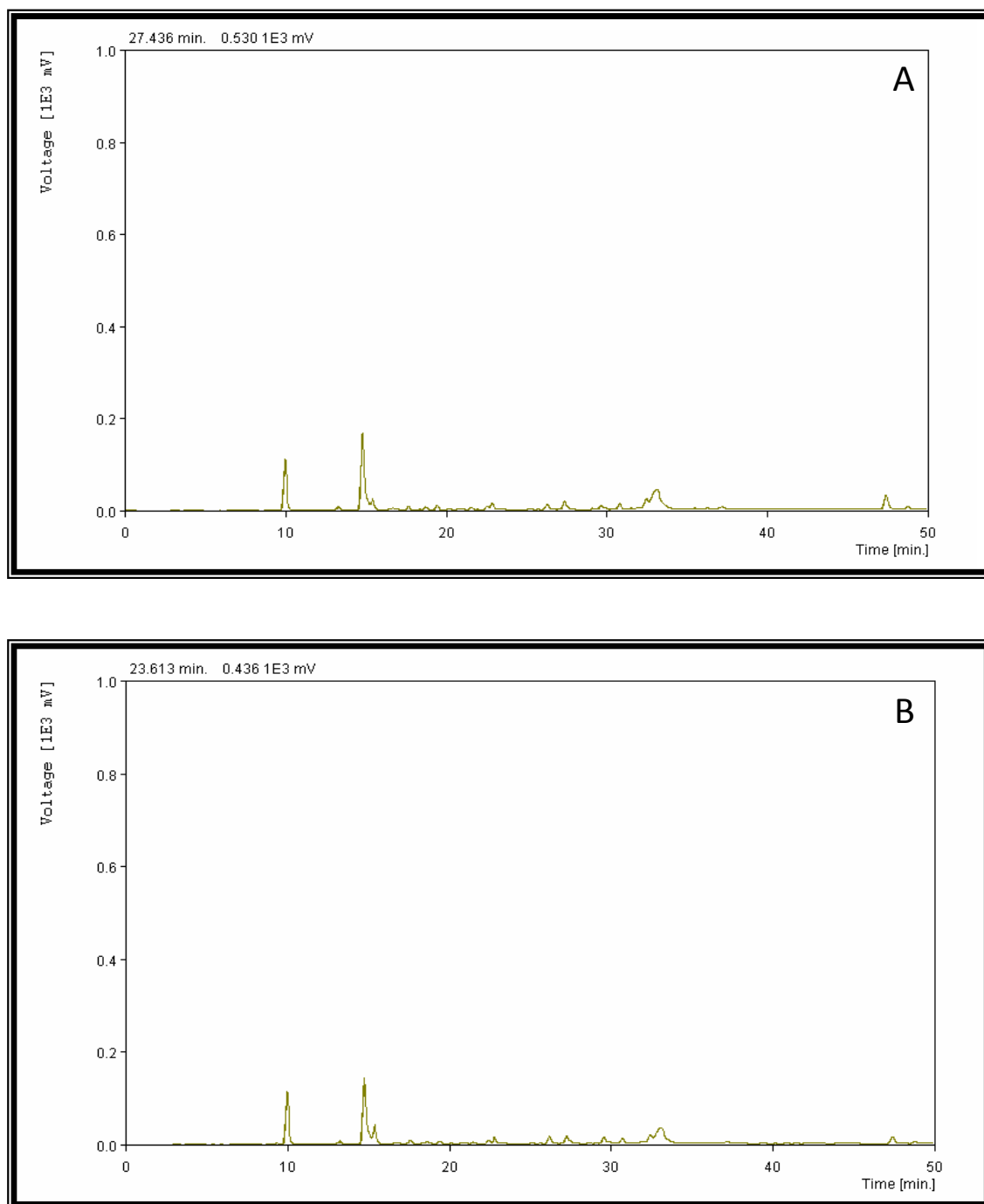


Fig 35F: HPLC analysis of total phenols from leaves of KS 244 cultivar during flooding stress A: 5-day & B: 7-day

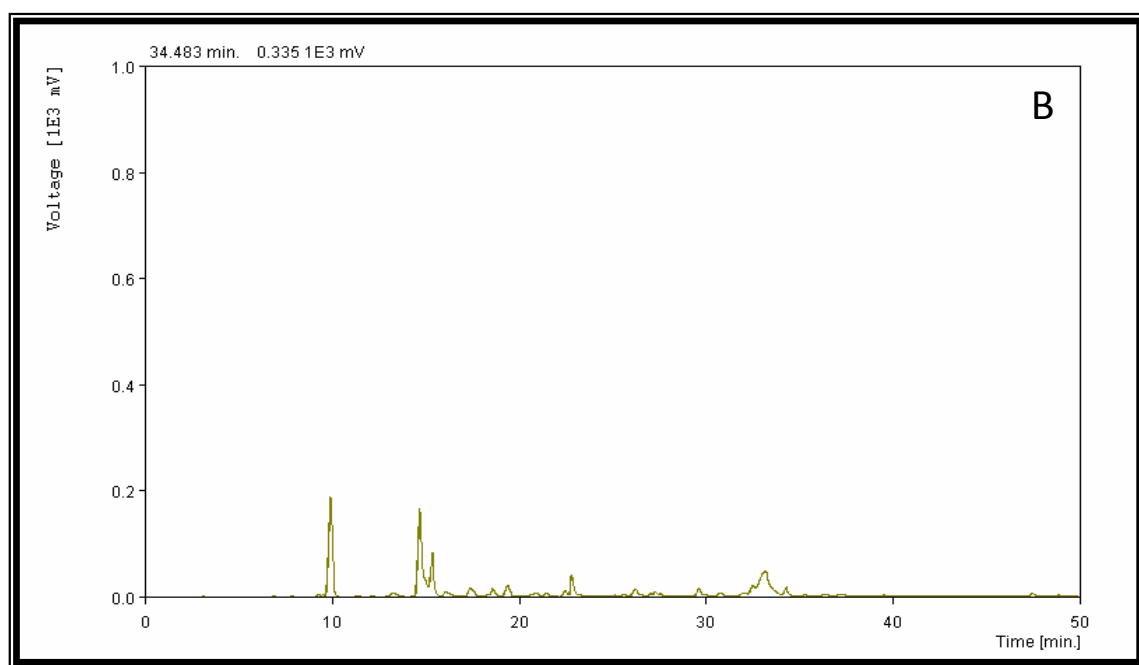
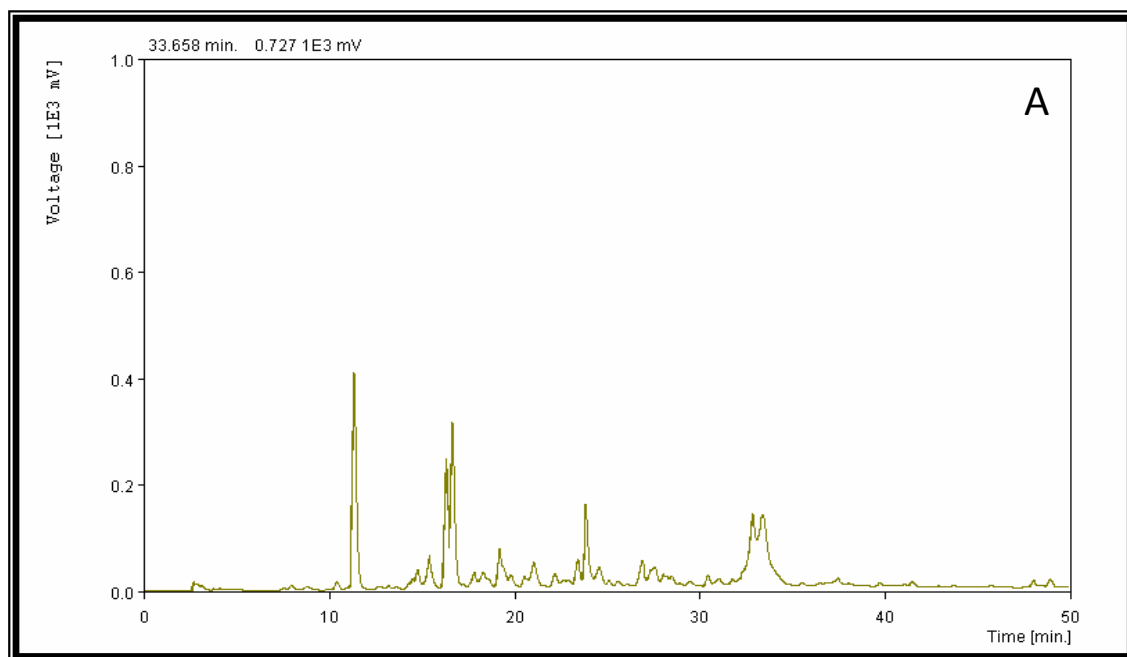


Fig 35G: HPLC analysis of total phenols from leaves of Dhanya cultivar during flooding stress A: 0-day & B: 3-day

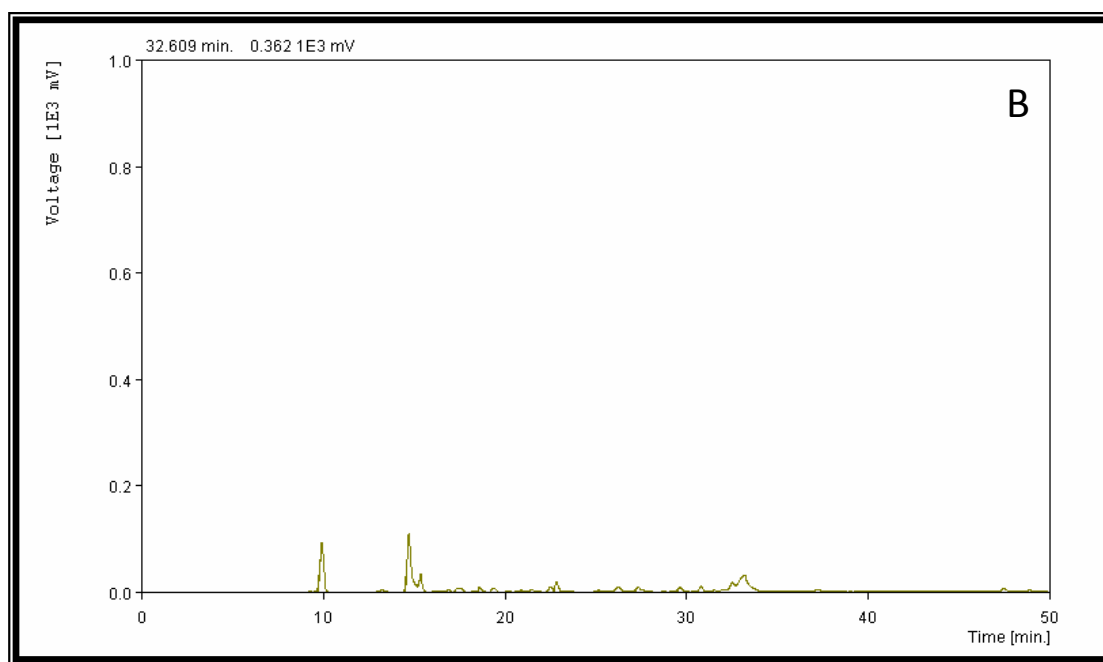
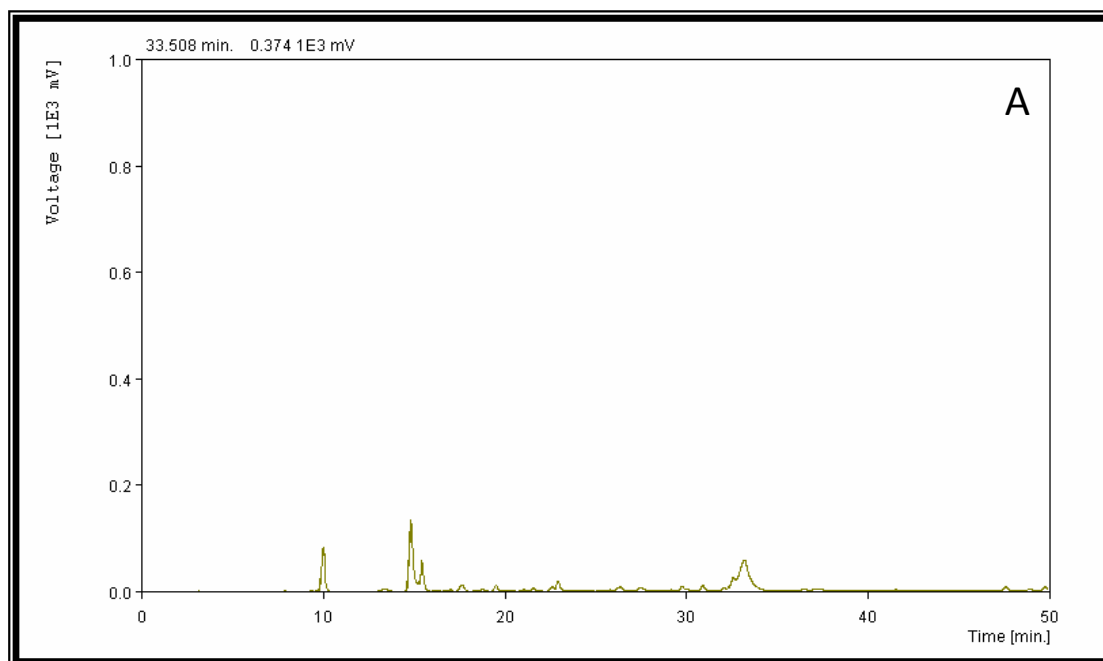


Fig 35H: HPLC analysis of total phenols from leaves of Dhanya cultivar during flooding stress A: 5-day & B: 7-day

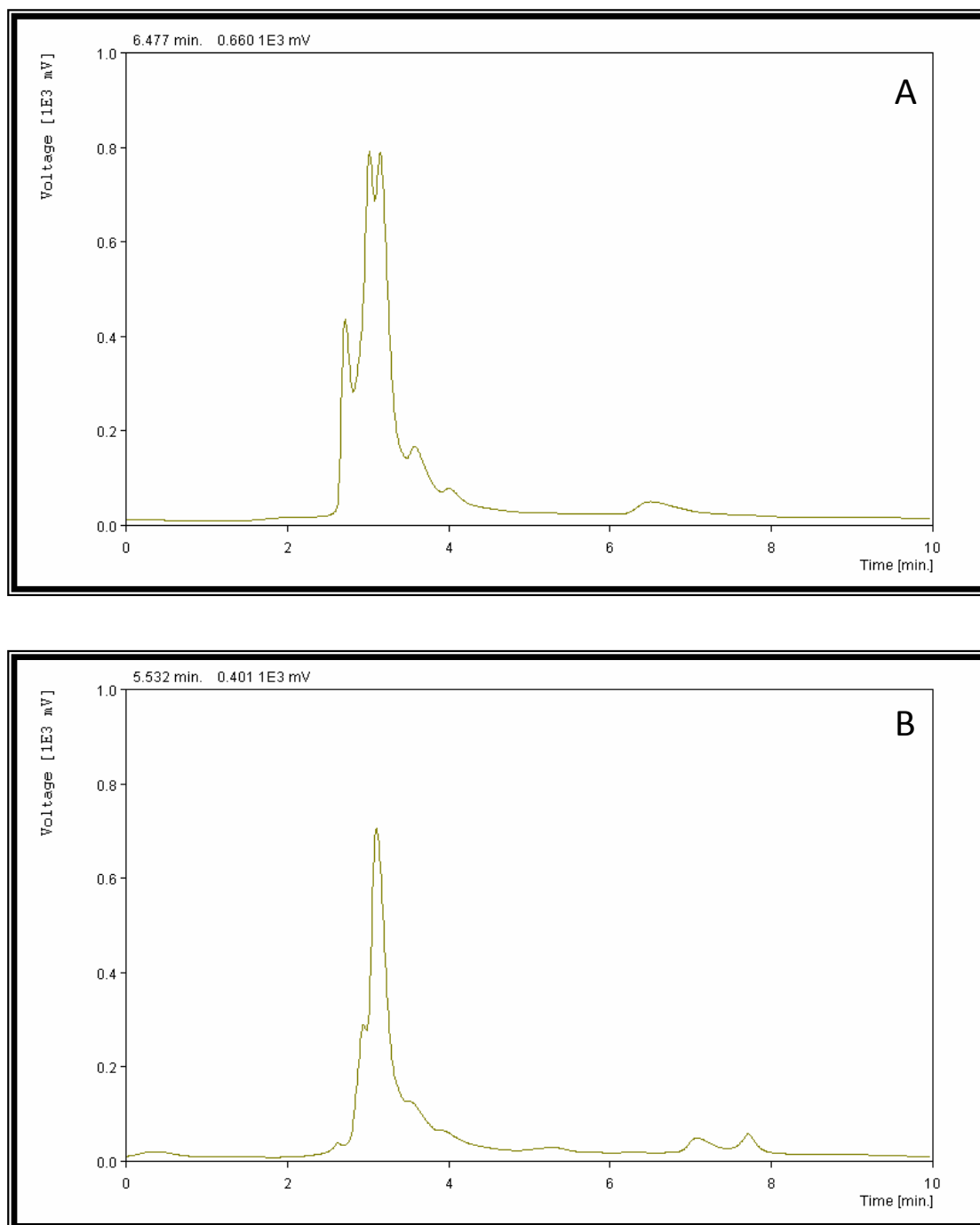


Fig 36A: HPLC of phenolic acids from leaves of KS 244 cultivar during drought stress A: 0-day & B: 3-day

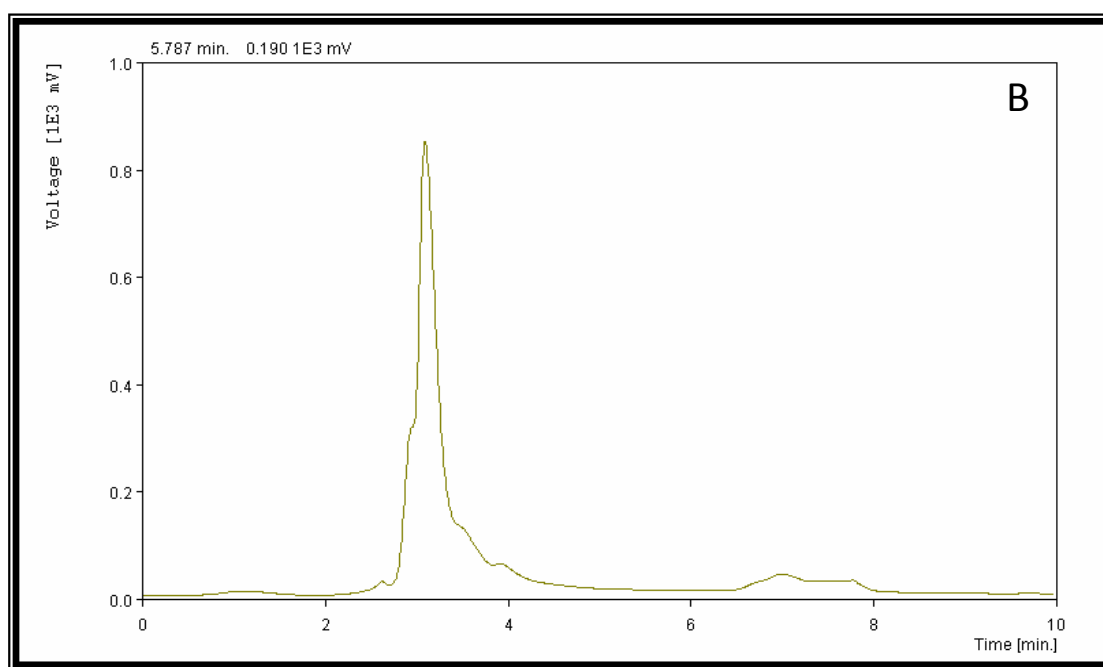
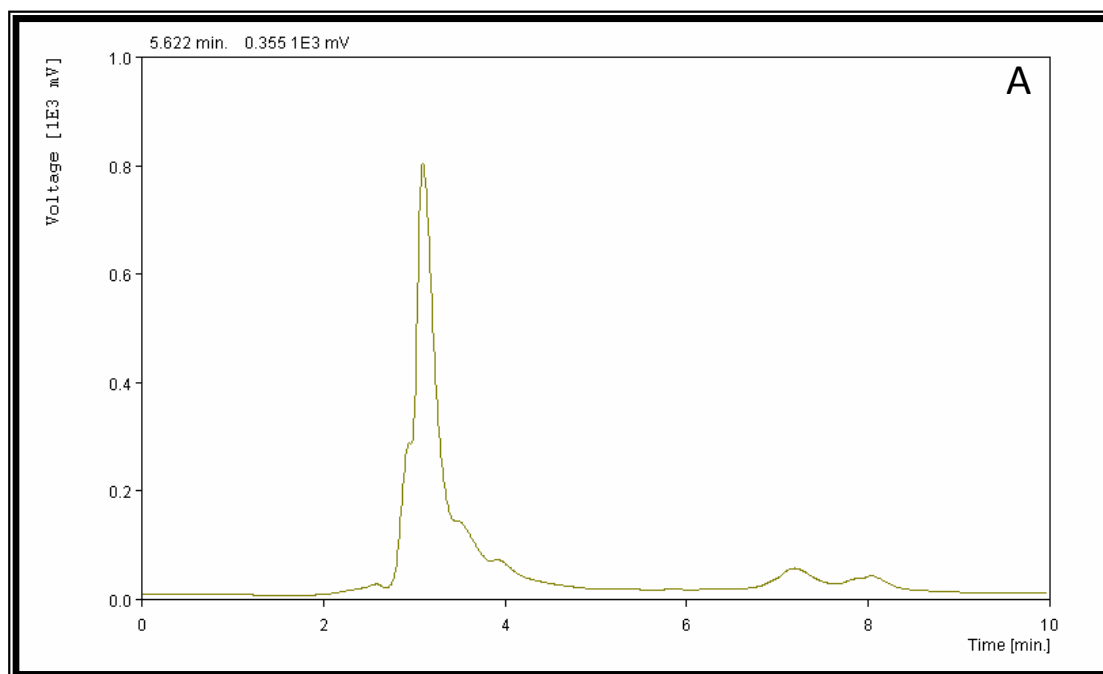


Fig 36B: HPLC of phenolic acids from leaves of KS 244 cultivar during drought stress A: 5-day & B: 7-day

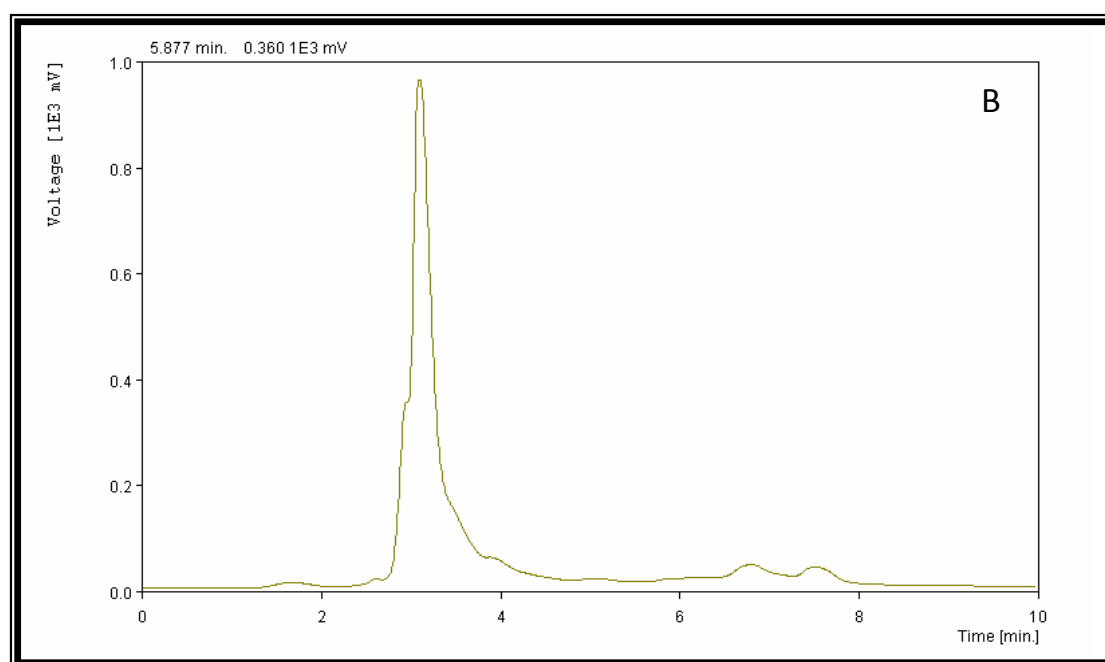
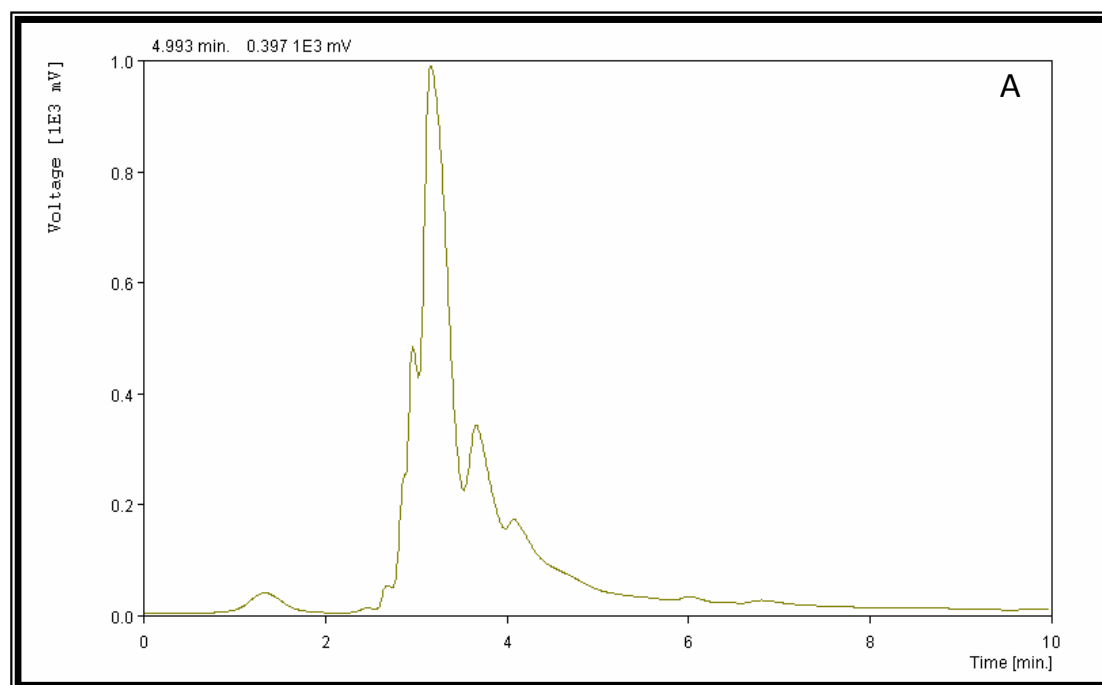


Fig 36C: HPLC of phenolic acids from leaves of Dhanya cultivar during drought stress A: 0-day & B: 3-day

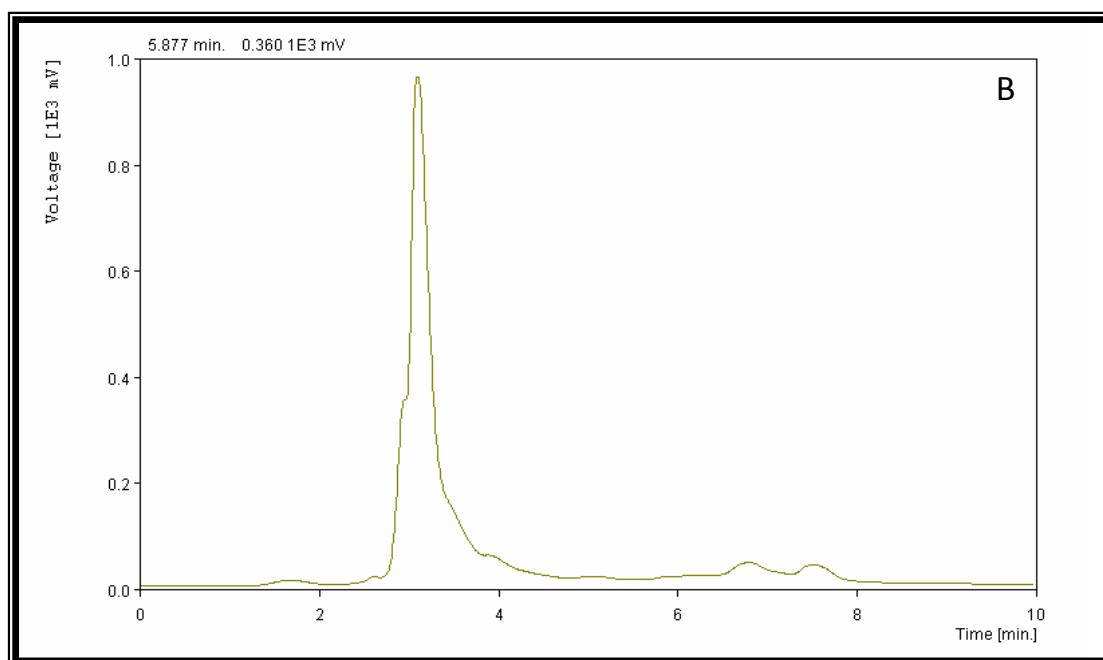
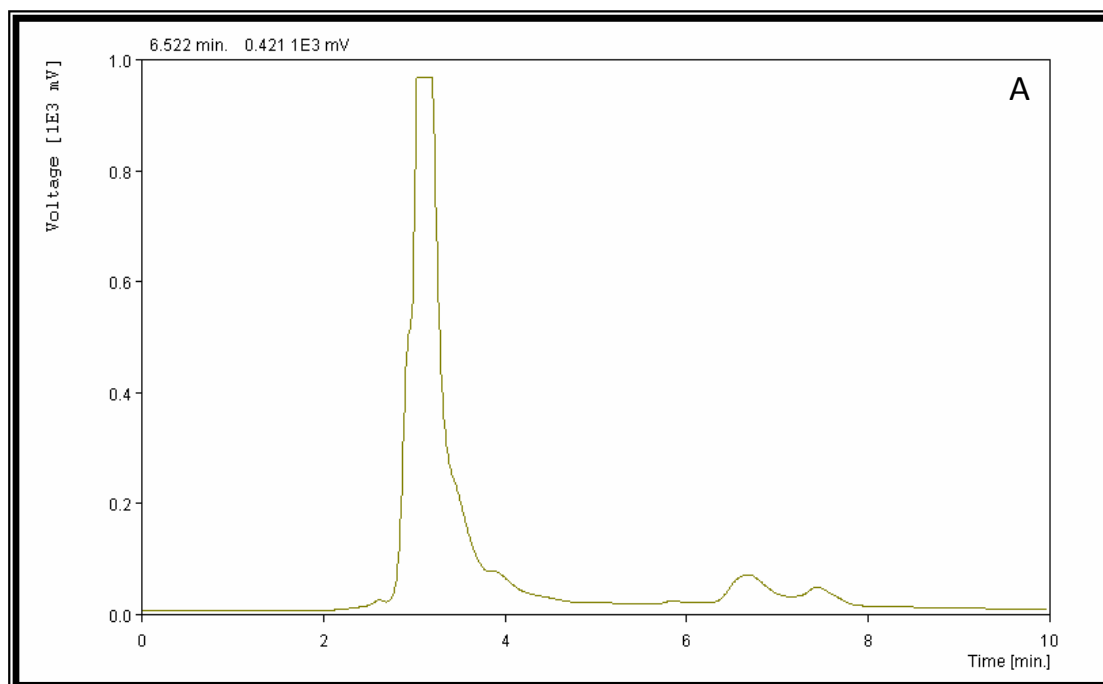


Fig 36D: HPLC of phenolic acids from leaves of Dhanya cultivar during drought stress A: 5-day & B: 7-day

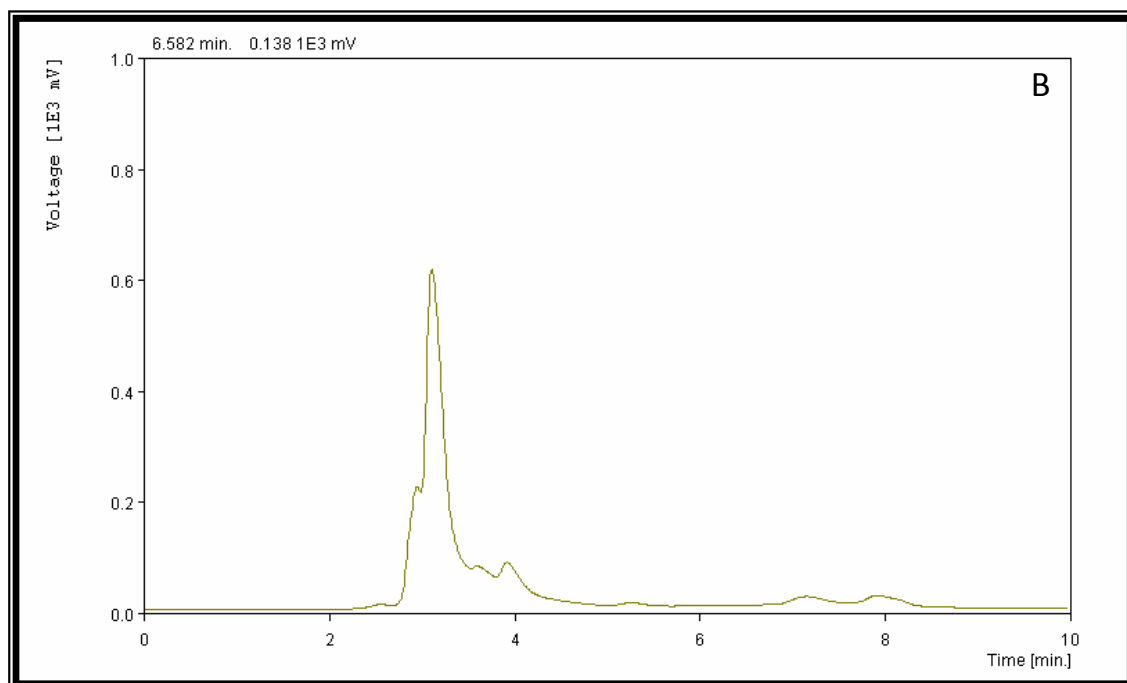
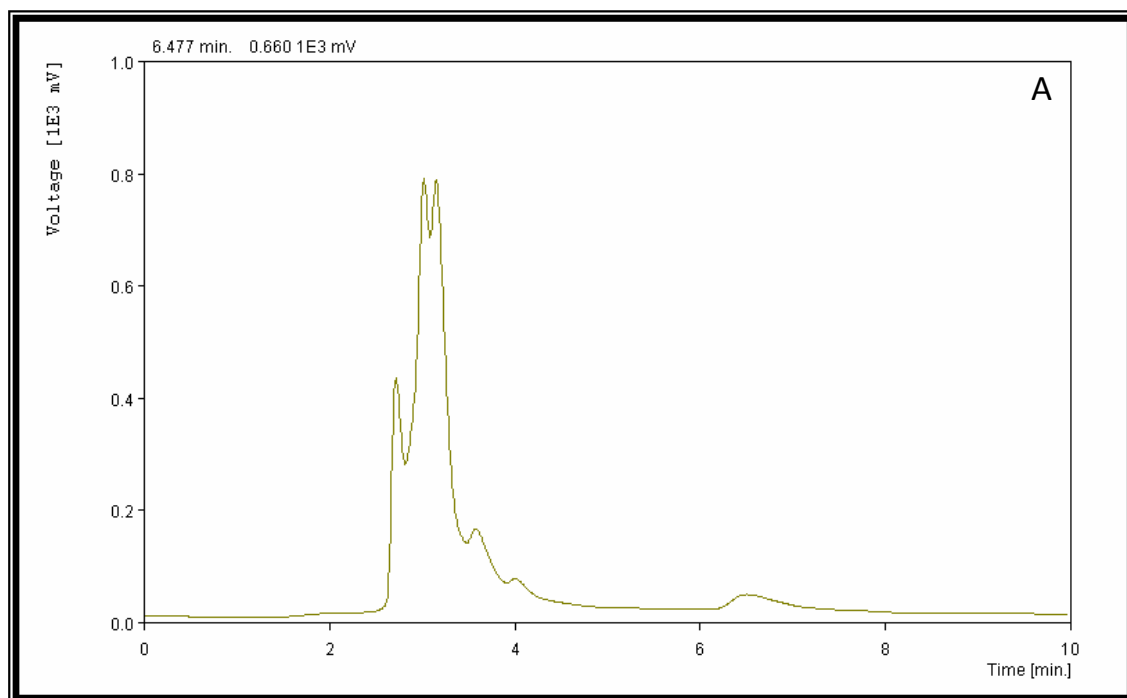


Fig 36E: HPLC of phenolic acids from leaves of KS 244 cultivar during flooding stress A: 0-day & B: 3-day

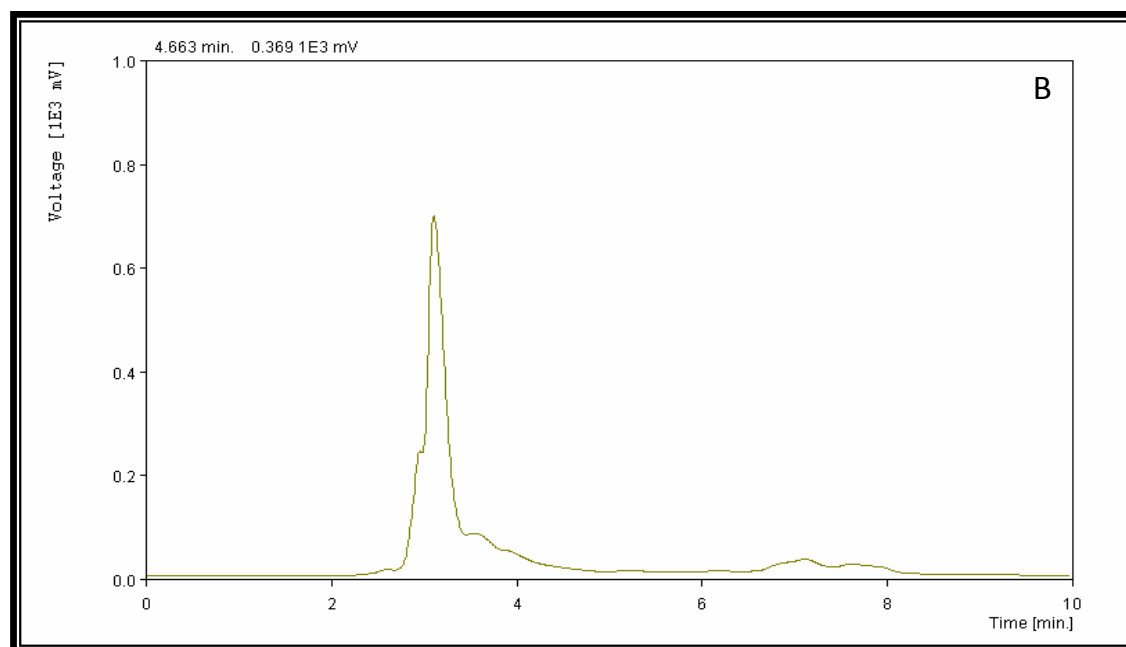
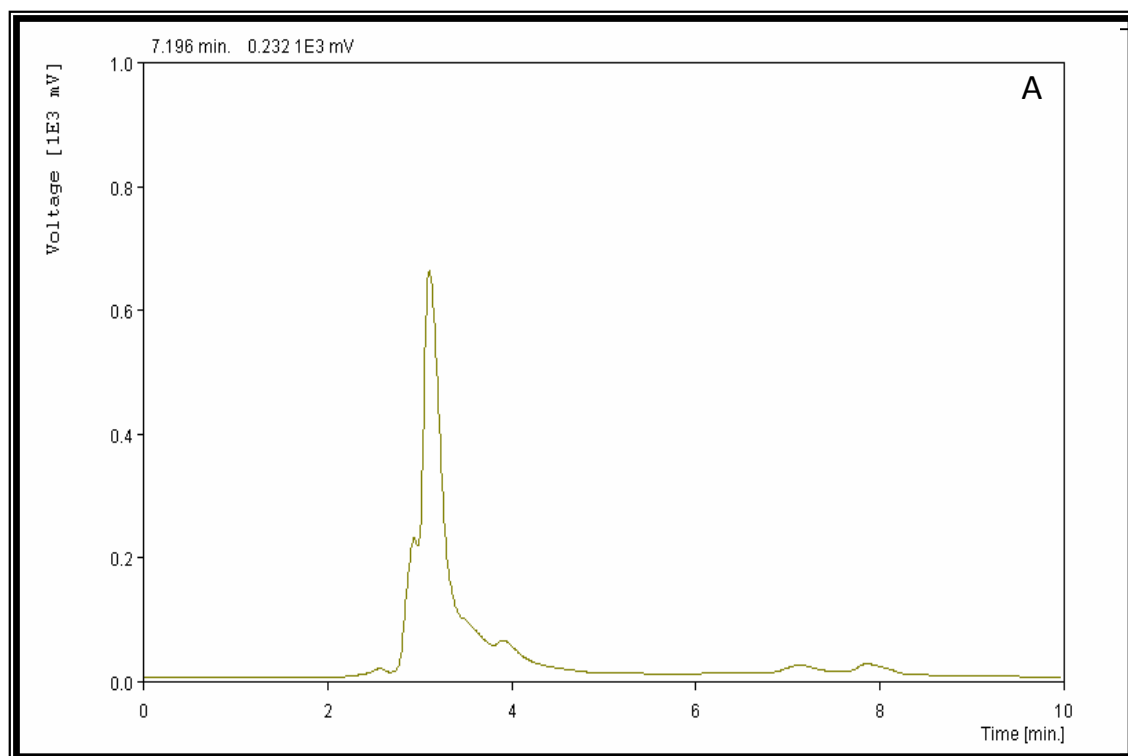


Fig 36F: HPLC of phenolic acids from leaves of KS 244 cultivar during flooding stress A: 5-day & B: 7-day

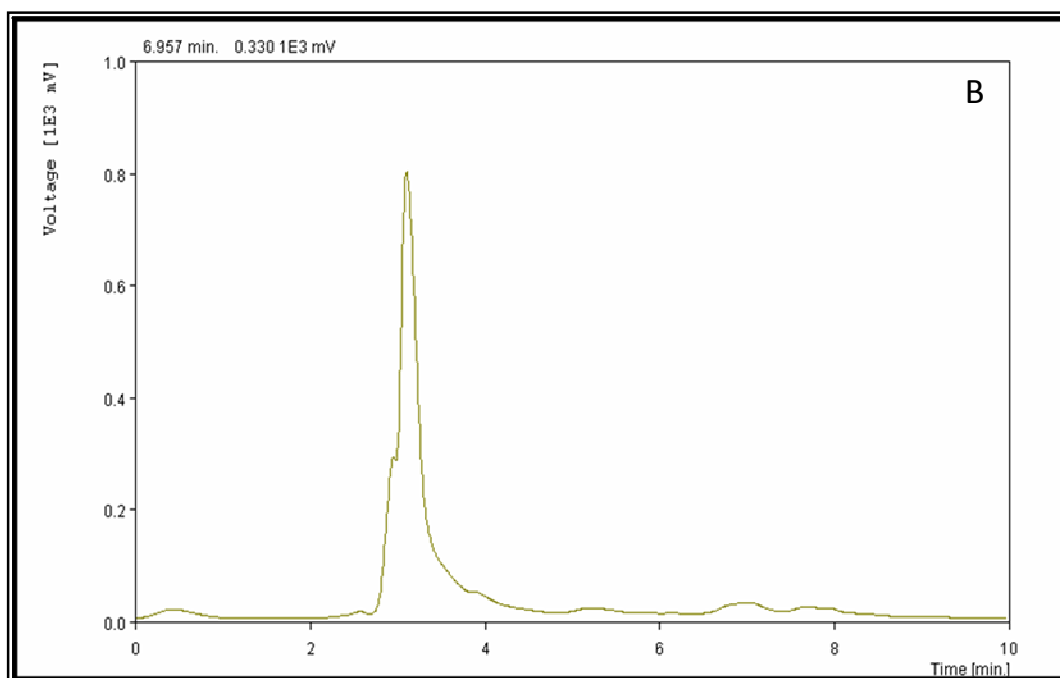
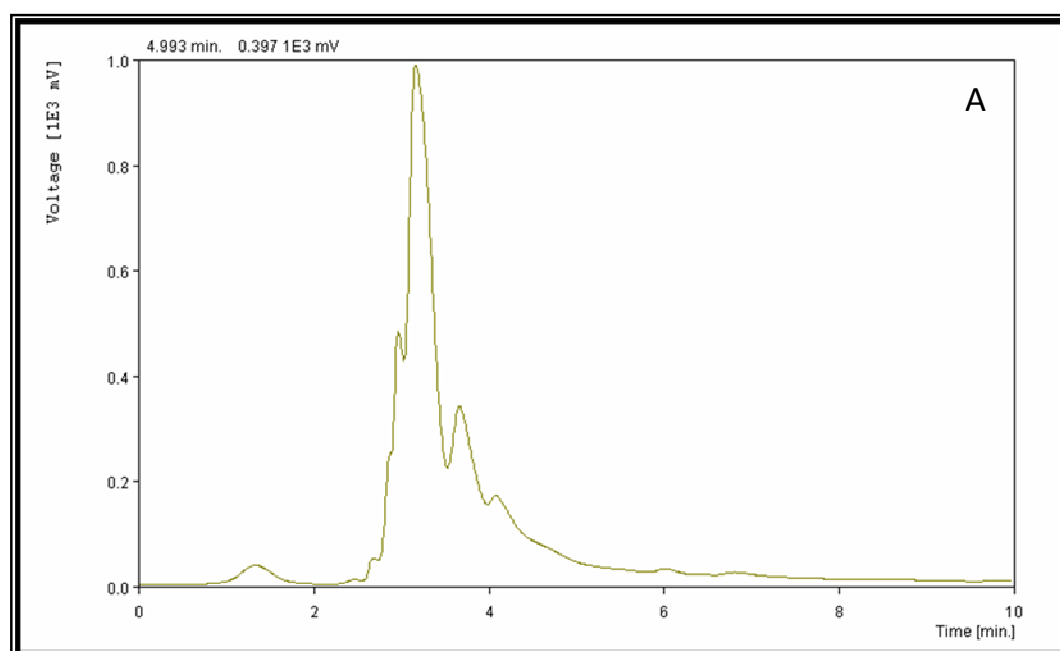


Fig 36G: HPLC of phenolic acids from leaves of Dhanya cultivar during flooding stress A: 0-day & B: 3-day

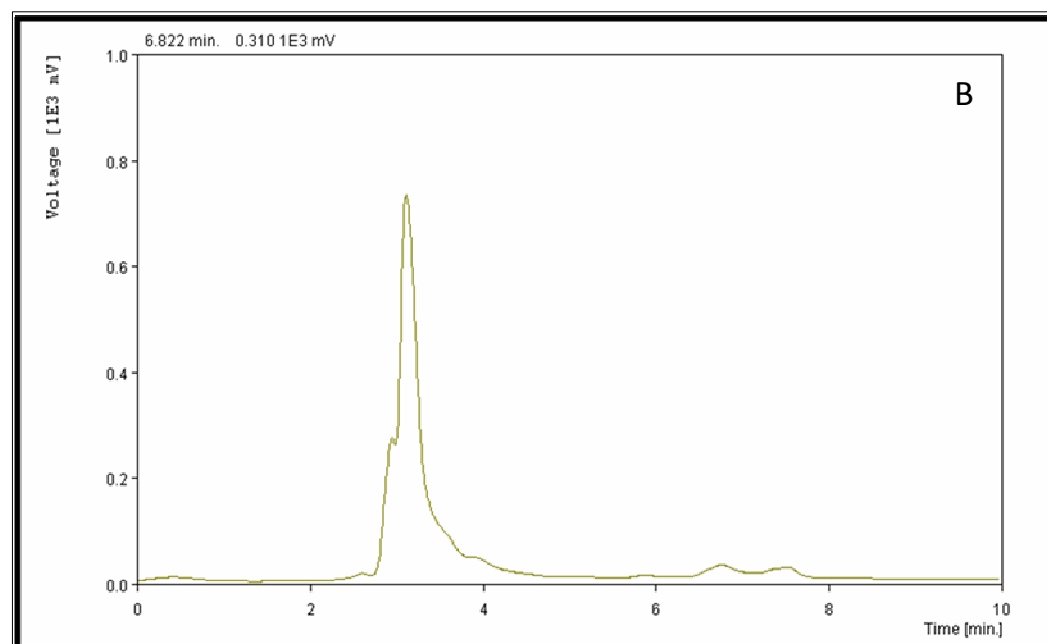
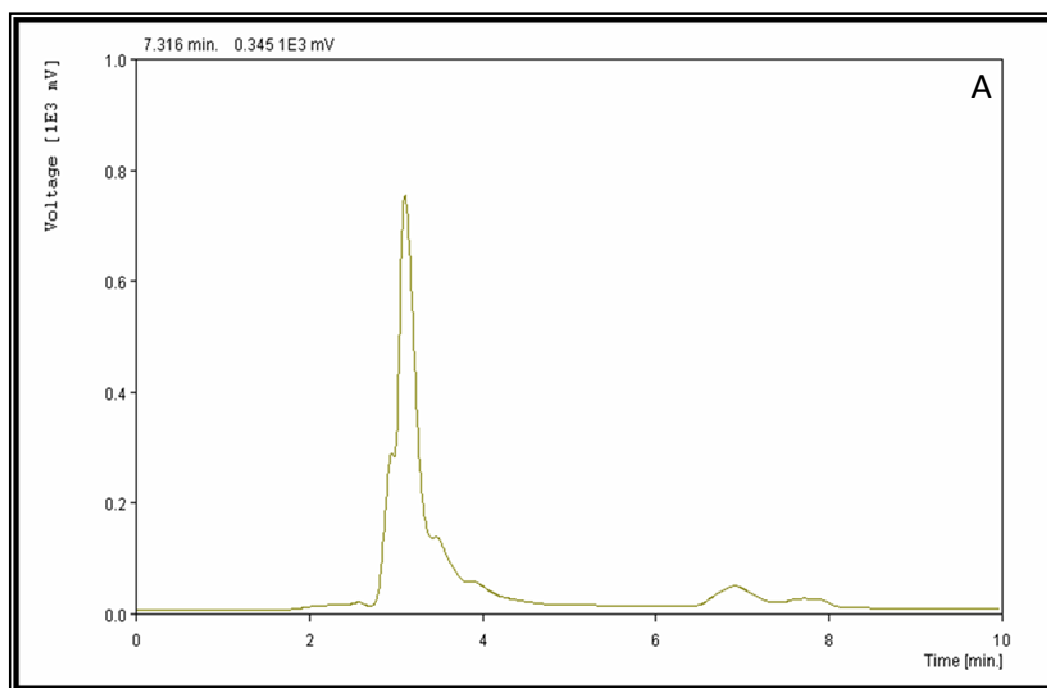


Fig 36H: HPLC of phenolic acids from leaves of Dhanya cultivar during flooding stress A: 5-day & B: 7-day

Table 35A: HPLC analysis of total phenol from leaves of KS 244 cultivar during drought stress *in vivo*

Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)	Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)
KS 244 0-day drought	1	9.700	0.46	KS 244	1	9.70	0.286
	2	13.15	0.065		2	13.77	0.013
	3	14.40	0.12		3	15.02	0.078
	4	15.65	0.10		4	15.96	0.065
	5	15.96	0.026		5	17.53	0.013
	6	17.53	0.052		6	18.45	0.026
	7	18.15	0.013		7	23.48	0.039
	8	19.42	0.026		8	27.23	0.039
	9	22.85	0.065		9	28.17	0.052
	10	26.29	0.026		10	31.61	0.026
	11	27.23	0.026		11	32.23	0.065
	12	29.74	0.013		12	32.87	0.091
	13	30.67	0.013	KS 244 5-day drought	1	9.70	0.221
	14	32.23	0.013		2	13.77	0.013
	15	37.56	0.013		3	15.02	0.208
	16	47.26	0.026		4	15.96	0.091
KS 244 5-day drought	1	9.70	0.023		5	18.45	0.013
	2	13.77	0.013		6	22.85	0.013
	3	15.02	0.065		7	23.48	0.026
	4	15.96	0.078		8	27.23	0.013
	5	18.45	0.026		9	38.17	0.013
	6	23.48	0.013		10	29.74	0.013
	7	27.23	0.013		11	31.61	0.013
	8	38.17	0.013		12	32.23	0.065
	9	29.74	0.013		13	32.87	0.091
	10	31.61	0.013		14	47.26	0.026
	11	32.23	0.065	KS 244 7-day drought	1	9.70	0.221
	12	32.87	0.078		2	13.77	0.013
	13	47.26	0.026		3	15.02	0.208
KS 244 7-day drought	1	9.70	0.023		4	15.96	0.091
	2	13.77	0.013		5	18.45	0.013
	3	15.02	0.065		6	22.85	0.013
	4	15.96	0.078		7	23.48	0.026
	5	18.45	0.026		8	27.23	0.013
	6	23.48	0.013		9	38.17	0.013
	7	27.23	0.013		10	29.74	0.013
	8	38.17	0.013		11	31.61	0.013
	9	29.74	0.013		12	32.23	0.065
	10	31.61	0.013		13	32.87	0.091
	11	32.23	0.065		14	47.26	0.026
	12	32.87	0.078				
	13	47.26	0.026				

Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)	Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)
Dhanya 0-day drought	1	2.50	0.013	Dhanya 3-day drought	1	7.51	0.013
	2	9.70	0.013		2	10.96	0.416
	3	10.96	0.520		3	15.02	0.091
	4	14.40	0.052		4	16.59	0.520
	5	15.02	0.091		5	17.53	0.026
	6	15.96	0.325		6	18.15	0.039
	7	16.59	0.403		7	19.42	0.033
	8	17.53	0.039		8	20.97	0.078
	9	18.15	0.039		9	21.91	0.065
	10	19.42	0.104		10	23.48	0.065
	11	20.97	0.065		11	23.79	0.169
	12	21.91	0.039		12	26.29	0.078
	13	23.48	0.078		13	38.17	0.065
	14	23.97	0.208		14	29.74	0.026
	15	23.41	0.065		15	32.23	0.247
	16	26.29	0.078		16	32.87	0.208
	17	27.23	0.065		17	35.68	0.026
	18	30.67	0.039		18	37.25	0.065
	19	30.99	0.039				
	20	32.23	0.182				
	21	33.18	0.182				
	22	47.26	0.039				
	23	47.89	0.039				
Dhanya 5-day drought	1	9.70	0.351	Dhanya 7-day drought	1	10.96	0.208
	2	13.15	0.026		2	14.40	0.013
	3	14.40	0.416		3	15.96	0.169
	4	15.02	0.403		4	16.59	0.091
	5	17.53	0.039		5	23.48	0.052
	6	18.15	0.026		6	26.29	0.013
	7	18.78	0.013		7	27.23	0.013
	8	19.42	0.026		8	32.23	0.039
	9	20.91	0.039		9	33.18	0.065
	10	23.48	0.091				
	11	26.29	0.039				
	12	27.23	0.039				
	13	30.67	0.039				
	14	30.99	0.039				
	15	32.23	0.117				
	16	33.18	0.156				
	17	47.89	0.026				

Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)	Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)
Dhanya 0-day drought	1	2.50	0.013	Dhanya 3-day drought	1	7.51	0.013
	2	9.70	0.013		2	10.96	0.416
	3	10.96	0.520		3	15.02	0.091
	4	14.40	0.052		4	16.59	0.520
	5	15.02	0.091		5	17.53	0.026
	6	15.96	0.325		6	18.15	0.039
	7	16.59	0.403		7	19.42	0.033
	8	17.53	0.039		8	20.97	0.078
	9	18.15	0.039		9	21.91	0.065
	10	19.42	0.104		10	23.48	0.065
	11	20.97	0.065		11	23.79	0.169
	12	21.91	0.039		12	26.29	0.078
	13	23.48	0.078		13	38.17	0.065
	14	23.97	0.208		14	29.74	0.026
	15	23.41	0.065		15	32.23	0.247
	16	26.29	0.078		16	32.87	0.208
	17	27.23	0.065		17	35.68	0.026
	18	30.67	0.039		18	37.25	0.065
	19	30.99	0.039	Dhanya 7-day drought	1	10.96	0.208
	20	32.23	0.182		2	14.40	0.013
	21	33.18	0.182		3	15.96	0.169
	22	47.26	0.039		4	16.59	0.091
	23	47.89	0.039		5	23.48	0.052
Dhanya 5-day drought	1	9.70	0.351		6	26.29	0.013
	2	13.15	0.026		7	27.23	0.013
	3	14.40	0.416		8	32.23	0.039
	4	15.02	0.403		9	33.18	0.065
	5	17.53	0.039				
	6	18.15	0.026				
	7	18.78	0.013				
	8	19.42	0.026				
	9	20.91	0.039				
	10	23.48	0.091				
	11	26.29	0.039				
	12	27.23	0.039				
	13	30.67	0.039				
	14	30.99	0.039				
	15	32.23	0.117				
	16	33.18	0.156				
	17	47.89	0.026				

Table 35C: HPLC analysis of total phenol from leaves of KS 244 during flooding stress *in vivo*

Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)	Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)
KS 244 0-day flooding	1	9.700	0.46	KS 244	1	9.70	0.273
	2	13.15	0.065		2	13.15	0.026
	3	14.40	0.12		3	41.40	0.286
	4	15.65	0.10		4	17.53	0.039
	5	15.96	0.026		5	18.15	0.013
	6	17.53	0.052		6	19.42	0.026
	7	18.15	0.013		7	22.85	0.039
	8	19.42	0.026		8	26.29	0.026
	9	22.85	0.065		9	27.23	0.013
	10	26.29	0.026		10	29.74	0.026
	11	27.23	0.026		11	30.67	0.013
	12	29.74	0.013		12	32.23	0.143
	13	30.67	0.013		13	47.26	0.026
	14	32.23	0.013	KS 244 5-day flooding	1	9.70	0.156
	15	37.56	0.013		2	13.15	0.013
	16	47.26	0.026		3	14.40	0.221
KS 244 5-day flooding	1	9.70	0.156		4	22.85	0.026
	2	13.15	0.013		5	26.29	0.013
	3	14.40	0.221		6	27.23	0.026
	4	22.85	0.026		7	29.74	0.013
	5	26.29	0.013		8	30.65	0.013
	6	27.23	0.026		9	32.23	0.065
	7	29.74	0.013		10	47.26	0.039
	8	30.65	0.013	KS 244 7-day flooding	1	9.70	0.143
	9	32.23	0.065		2	14.40	0.182
	10	47.26	0.039		3	15.65	0.039
KS 244 7-day flooding	1	9.70	0.156		4	26.29	0.026
	2	13.15	0.013		5	27.23	0.026
	3	14.40	0.221		6	29.74	0.026
	4	22.85	0.026		7	30.67	0.013
	5	26.29	0.013		8	32.23	0.052
	6	27.23	0.026		9	47.26	0.026
	7	29.74	0.013				
	8	30.65	0.013				
	9	32.23	0.065				
	10	47.26	0.039				

Table 35D: HPLC analysis of total phenol from leaves of Dhanya maize cultivar during flooding stress *in vivo*

Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)	Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)
Dhanya 0-day flooding	1	2.50	0.013	Dhanya	1	9.70	0.234
	2	9.70	0.013		2	14.40	0.208
	3	10.96	0.520		3	15.96	0.117
	4	14.40	0.052		4	17.23	0.026
	5	15.02	0.091		5	18.15	0.026
	6	15.96	0.325		6	19.42	0.026
	7	16.59	0.403		7	23.48	0.052
	8	17.53	0.039		8	26.29	0.013
	9	18.15	0.039		9	29.74	0.013
	10	19.42	0.104	3-day flooding	10	32.87	0.065
	11	20.97	0.065		11	34.70	0.013
	12	21.91	0.039				
	13	23.48	0.078				
	14	23.97	0.208				
	15	23.41	0.065				
	16	26.29	0.078				
	17	27.23	0.065				
	18	30.67	0.039				
	19	30.99	0.039				
	20	32.23	0.182				
	21	33.18	0.182				
	22	47.26	0.039				
	23	47.89	0.039				
Dhanya 5-day flooding	1	10.96	0.104	Dhanya	1	9.70	0.130
	2	15.96	0.182		2	14.40	0.143
	3	16.59	0.078		3	15.02	0.039
	4	17.53	0.013		4	17.53	0.013
	5	19.42	0.013		5	18.78	0.013
	6	23.48	0.026		6	19.42	0.013
	7	26.29	0.013	7-day flooding	7	26.29	0.026
	8	27.23	0.013		8	33.18	0.039
	9	29.74	0.013				
	10	30.99	0.013				
	11	33.18	0.078				

Table 36A: HPLC analysis of phenolic acids from leaves of KS 244 cultivar during drought stress *in vivo*

Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)	Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)
KS 244 0-day drought	1	2.73	0.43	KS 244 3-day drought	1	2.60	0.05
	2	3.10	0.77		2	2.98	0.28
	3	3.22	0.77		3	3.22	0.70
	4	3.72	0.16		4	7.13	0.05
	5	4.63	0.09		5	7.75	0.06
	6	6.51	0.05				
KS 244 5-day drought	1	3.22	0.80	KS 244 7-day drought	1	3.22	0.83
	2	7.13	0.05		2	7.13	0.05
	3	8.06	0.04				

Table 36B: HPLC analysis of phenolic acids from the leaves of Dhanya cultivar during drought stress *in vivo*

Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)	Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)
Dhanya 0-day drought	1	1.36	0.04	Dhanya 3-day drought	1	3.22	0.92
	2	2.98	0.48		2	6.82	0.06
	3	3.22	0.97		3	7.44	0.04
	4	3.72	0.33	Dhanya 7-day drought	1	3.22	0.95
	5	4.03	0.16		2	6.82	0.07
Dhanya 5-day drought	1	3.22	0.90		3	7.44	0.06
	2	6.82	0.05				
	3	7.44	0.04				

Table 36C: HPLC analysis of phenolic acids from leaves of KS 244 during flooding stress *in vivo*

Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)	Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)
KS 244 0-day flooding	1	2.73	0.43	KS 244 3-day flooding	1	2.98	0.22
	2	3.10	0.77		2	3.22	0.62
	3	3.22	0.77		3	3.60	0.09
	4	3.72	0.16		4	4.03	0.08
	5	4.63	0.09		5	7.13	0.03
	6	6.51	0.05		6	8.06	0.03
KS 244 5-day flooding	1	2.98	0.23	KS 244 7-day flooding	1	3.22	0.69
	2	3.22	0.65		2	7.13	0.04
	3	4.03	0.07				

Table 36D: HPLC analysis of phenolic acids from the leaves of Dhanya cultivar during flooding stress *in vivo*

Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)	Cultivar and Treatment	No.of peaks	Retention time (minutes)	Voltage (mV)
Dhanya 0-day flooding	1	1.36	0.04	Dhanya 3-day flooding			
	2	2.98	0.48		1	3.22	0.80
	3	3.22	0.97		2	6.82	0.05
	4	3.72	0.33				
	5	4.03	0.16				
Dhanya 5-day flooding	1	3.22	0.74	Dhanya 7-day flooding	1	3.22	0.74
	2	6.82	0.05		2	6.82	0.04

4.15. Changes in biochemical constituents of maize following induction of water stress in the field during reproductive stages

The earlier experiments were mainly conducted on water stress applied to seedlings at early vegetative growth. However, water stress during later stages of growth- ie., during reproductive stage might have different effects on metabolism in the plants. In order to determine this, a set of experiments were further carried out with 2 of the cultivars- KS 244 and Dhanya. Among the two cultivars, Dhanya showed more severe signs of wilting, while KS 244 could withstand drought of up to 20 days (Fig 37).

4.15.1. Protein and antioxidative enzymes

Protein content in the leaves of the two test cultivars increased during drought stress and a greater increase was seen in KS 244 cultivar. The activity of two antioxidative enzymes - peroxidase (POX) and catalase (CAT) were determined in two cultivars of maize during drought stress in the field condition. It was seen that in case of Dhanya there was a decrease in the activity of both POX and CAT on the 20th day of drought stress, but unlike Dhanya the other test cultivar KS 244 showed an opposite trend, where the activity of both POX and CAT increased during the period of drought stress (Table 37).

4.15.2. Hydrogen peroxide

In Dhanya the H₂O₂ accumulation on the 20th day of drought stress was almost double of that on 0-day of stress. But in case of KS 244 the H₂O₂ accumulation was seen to decrease on the 20th day of stress (Table 37).

4.15.3. Proline and Carbohydrate content

There was an increase in proline content in the leaves and root of two cultivars of maize during drought stress. The proline content was more in the leaves than in the roots of the test plant, and was greater in KS 244 than in Dhanya during drought stress (Fig 38). Total sugar and reducing sugar also showed an increase during period of stress in both the cultivars but unlike proline content the sugar content was greater in the roots of the plants than the leaves, but like proline content the total sugar was much more in KS 244 than in Dhanya on the 20th day of drought stress (Fig 39A & 39B).

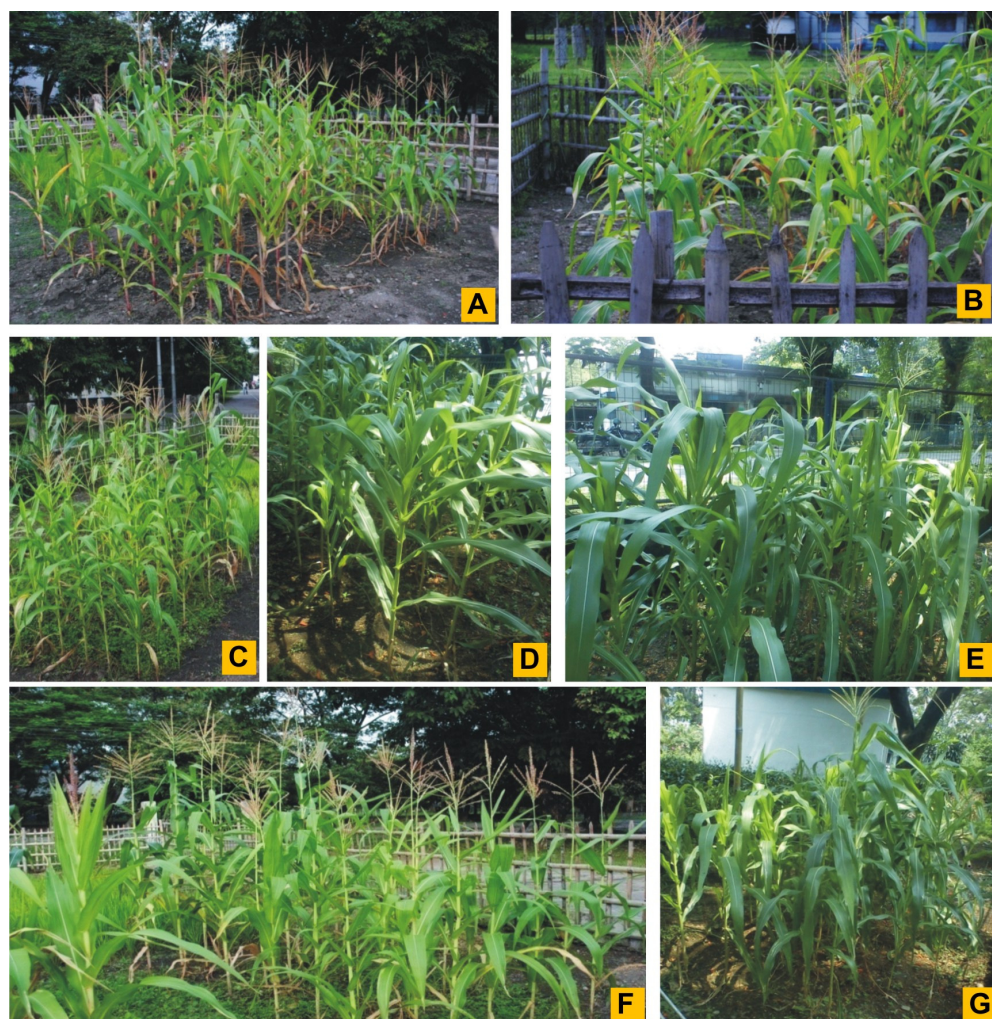


Fig. 37: Pictures of maize field - A,B,C,F: control plants of Dhanya and KS 244.
D, E,G: drought subjected plants of Dhanya and KS 244 cultivar

Table 37: Protein content, catalase, peroxidase activity and hydrogen peroxide accumulation in leaves of two cultivars of maize during drought stress in the field

CULTIVARS TREATMENT	DHANYA		KS 244	
	0-DAY	20-DAY	0-DAY	20-DAY
PROTEIN (LEAF) mg g tissue FW⁻¹	8.67±0.204 ^a	33.33±0.770 ^b	7.16±0.160 ^a	48.44±1.176 ^a
CATALASE μmole H₂O₂ mg protein⁻¹ min⁻¹	1.87±0.373 ^a	0.961±0.096 ^a	1.89±0.471 ^a	2.43±0.258 ^a
PEROXIDASE mmole 0-dianisidine mg protein⁻¹ min⁻¹	15.56±2.175 ^a	10.89±1.124 ^a	20.47±1.863 ^a	28.81±0.018 ^a
HYDROGEN PEROXIDE μM g⁻¹ tissue FW	16.4±0.348 ^a	44.29±0.695 ^b	28.17±0.318 ^a	27.71±0.151 ^a

Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in Students 't' test within each cultivar.

4.15.4. Chlorophyll content

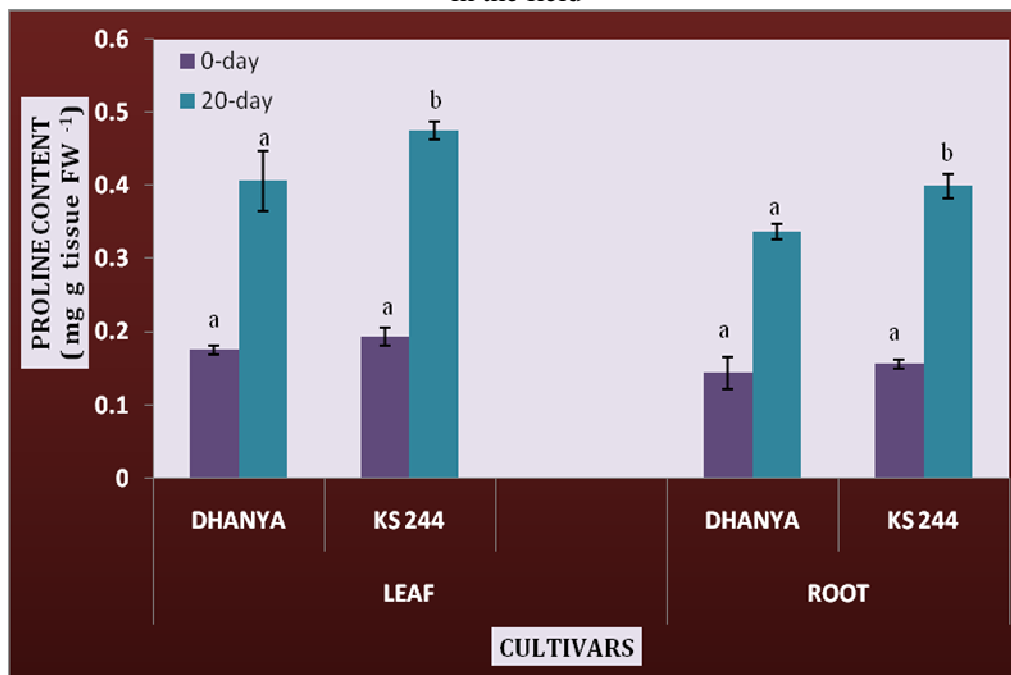
The chlorophyll content (Table 38) in both cultivars of maize decreased during drought stress in the field. A greater decrease in the chlorophyll content was seen in Dhanya cultivar than in KS 244.

Table 38: Chlorophyll content in leaves of two cultivars of maize during late drought stress

Cultivar	Treatment	Total chlorophyll Mg g tissue FW ⁻¹	Chlorophyll-a mg g tissue FW ⁻¹	Chlorophyll-b mg g tissue FW ⁻¹
DHANYA	0-day	7.62±0.059 ^a	5.00±0.087 ^a	2.63±0.047 ^a
	20-day	2.78±0.035 ^b	1.78±0.019 ^b	1.00±0.047 ^b
KS 244	0-day	7.31±0.125 ^a	4.90 ±0.157 ^a	2.41±0.064 ^a
	20-day	5.33±0.084 ^b	3.09±0.035 ^a	2.26±0.118 ^a

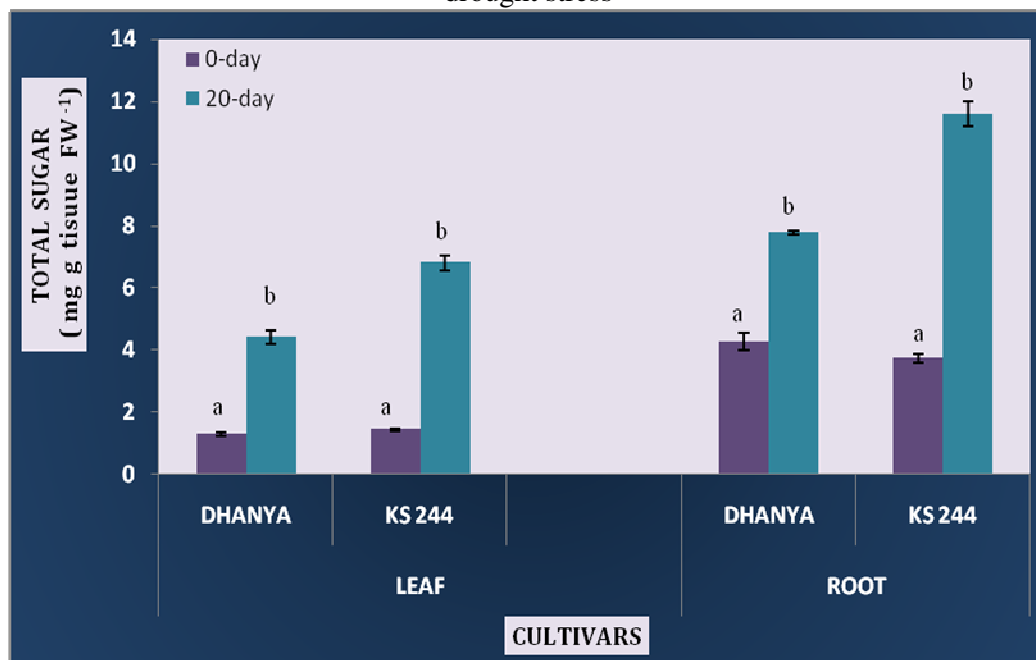
Average of three replicate experiments. ± =SE; Different superscripts indicate significant differences at P ≤0.05 in students 't' test within each cultivar.

Fig 38: Proline content leaves of two maize cultivars during drought stress in the field



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

Fig 39A: Total carbohydrate in leaves of two cultivars of maize during drought stress



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

4.15.5. Isozyme analysis of peroxidase

Isozyme analysis of peroxidase in the leaves of two cultivars during drought stress showed new bands of peroxidase on the 20th day of drought in both the cultivars (Table 39 and Fig 40)

Table 39: Relative mobility (R_m) value of peroxidase isozyme in leaves of Dhanya and KS 244 cultivar of maize during drought stress in the field.

Cultivar	Treatment	Lane Number	No. of isozyme bands	R_m value
DHANYA	0-day	1	5	0.26,0.38,0.49
DHANYA	0-day	2	5	0.26,0.38,0.49
DHANYA	20-day	3	7	0.08,0.17,0.26,0.38,0.49,0.58,0.66
KS 244	0-day	4	4	0.08,0.17,0.26,0.38
KS 244	0-day	5	4	0.08,0.17,0.26,0.38
KS 244	20-day	6	8	0.17,0.26,0.38,0.49,0.58,0.66,0.69,0.80

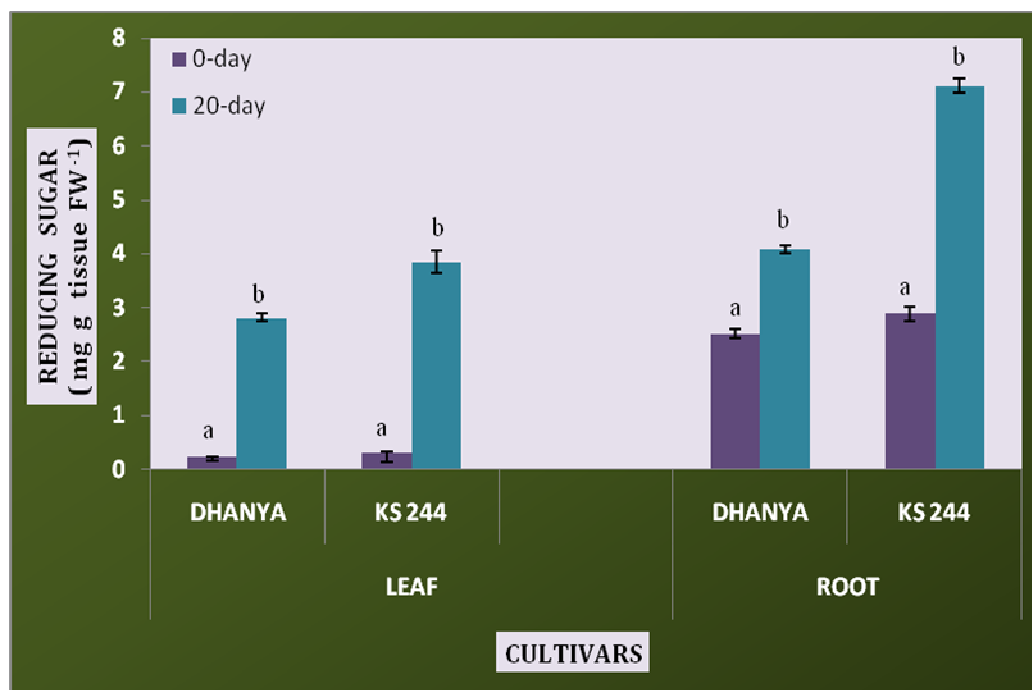
4.16. Effect of chemical pre-treatment followed by drought stress

Pre-treatment of the maize plants with ABA and proline increased the ability of the plants to withstand drought. Dhanya, which was generally susceptible, could also withstand stress to a greater degree (Figs 41 and 42)

4.16.1. Relative Water Content

Withholding of water in pots for seven days in two months old maize plants for inducing drought stress resulted in near wilting of the plants especially in case Dhanya cultivar. Relative water content in the leaves of (Fig 43) showed that there was a significant decrease in Dhanya than in KS 244 after 7days of drought stress, indicating KS 244 to be a much more tolerant cultivar than Dhanya. Foliar application of ABA and Proline improved the tolerance level in both the cultivars especially enhancing the tolerance level of the susceptible cultivar, making it 30 times more tolerant than when subjected to drought without any prior application of the chemicals. The best results were seen in case of the plant treated with 30mM of proline where RWC was 67.5% in the susceptible cultivar after drought stress, whereas the untreated plant under drought stress showed 34.23% of RWC.

Fig 39B: Reducing sugar in leaves of two cultivars of maize during late drought stress



Different letters above different bars indicate the significant differences at $P \leq 0.05$ Students 't' test within each cultivar

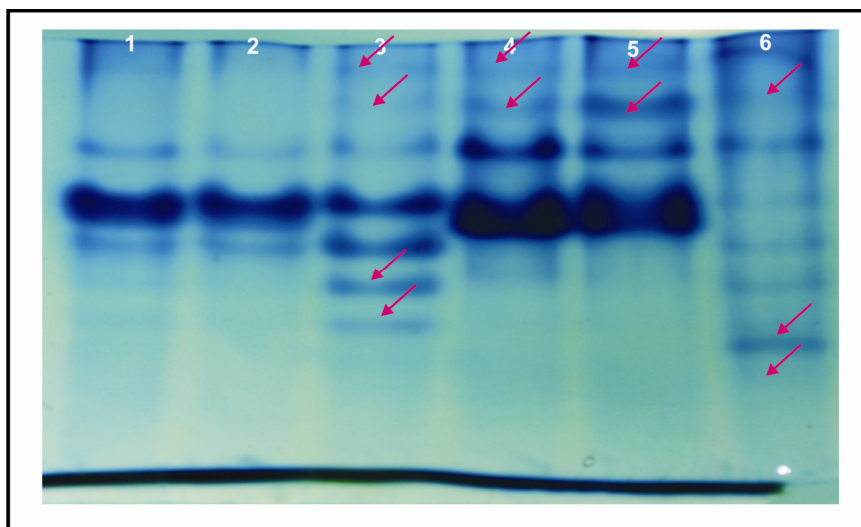


Fig 40: Analysis of peroxidase isozyme of Dhanya and KS 244 cultivar during drought stress in the field. Peroxidase isozyme -**Lane 1&2:** control of KS 244, **Lane 3:** 20-day drought of KS 244, **Lane 4&5:** control of Dhanya, **Lane 6:** 20-day drought of KS 244.

4.16.2. Membrane injury

Electrolytic leakage (Fig 44) was determined for the two cultivars- KS 244 and Dhanya after 7 day of drought stress where KS 244 exhibited values less than the susceptible cultivar Dhanya. But the plants treated with ABA and proline treatment prior to stress exhibited values less than the untreated plants subjected to drought stress. Maize plants under drought stress exhibited membrane lipid peroxidation, measured as MDA accumulation. Dhanya cultivar showed five times greater increase in lipid peroxidation than KS 244 during drought stress. Amelioration with ABA and proline resulted in the decrease of MDA content where again plants pre-treated with 30mmol of proline showed best results (Table 40).

4.16.3. Proline and Total soluble sugar accumulation

Proline (Fig 45) is considered to be one of the most important markers of drought stress and was seen to increase in the leaves of maize plant during the stress condition. More accumulation of proline was seen in the tolerant cultivar (KS 244) than in the susceptible cultivar (Dhanya) after 7 days of drought stress. Pre-treatment of both cultivars resulted in greater accumulation of proline during drought and best results were seen in plants treated with 30mmol of proline. Both the cultivars showed almost three fold increases in the proline content in plants treated with 30mmol of proline than the untreated plant subjected to drought stress. Similar results was seen in case of total soluble sugar content (Fig 46) where sugar content increased during drought stress in both cultivars, and amelioration of these two cultivars with ABA and proline resulted in further accumulation of carbohydrate content in the leaves of the two cultivars.

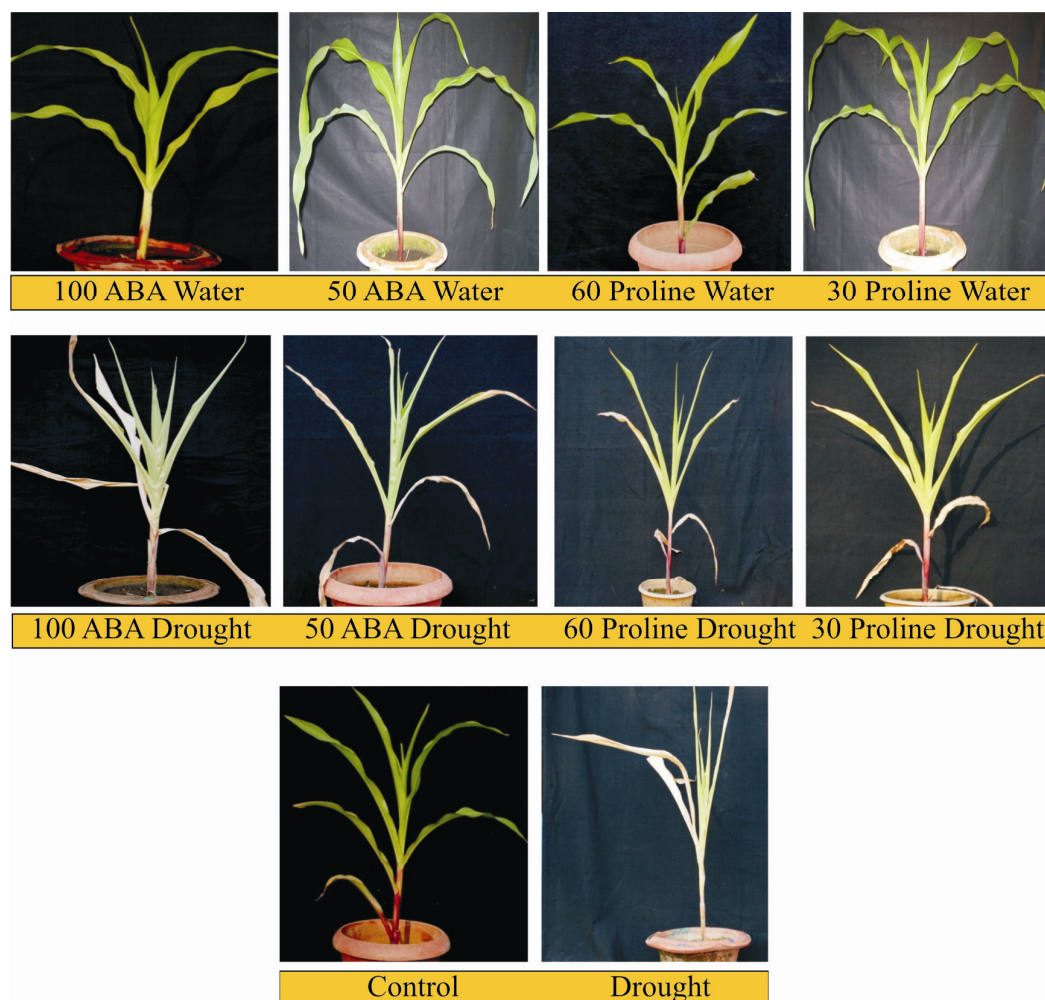


Fig 41: Amelioration of Dhanya cultivar by two chemicals abscisic acid (100 μ M and 50 μ M) and proline (60mM and 30mM)

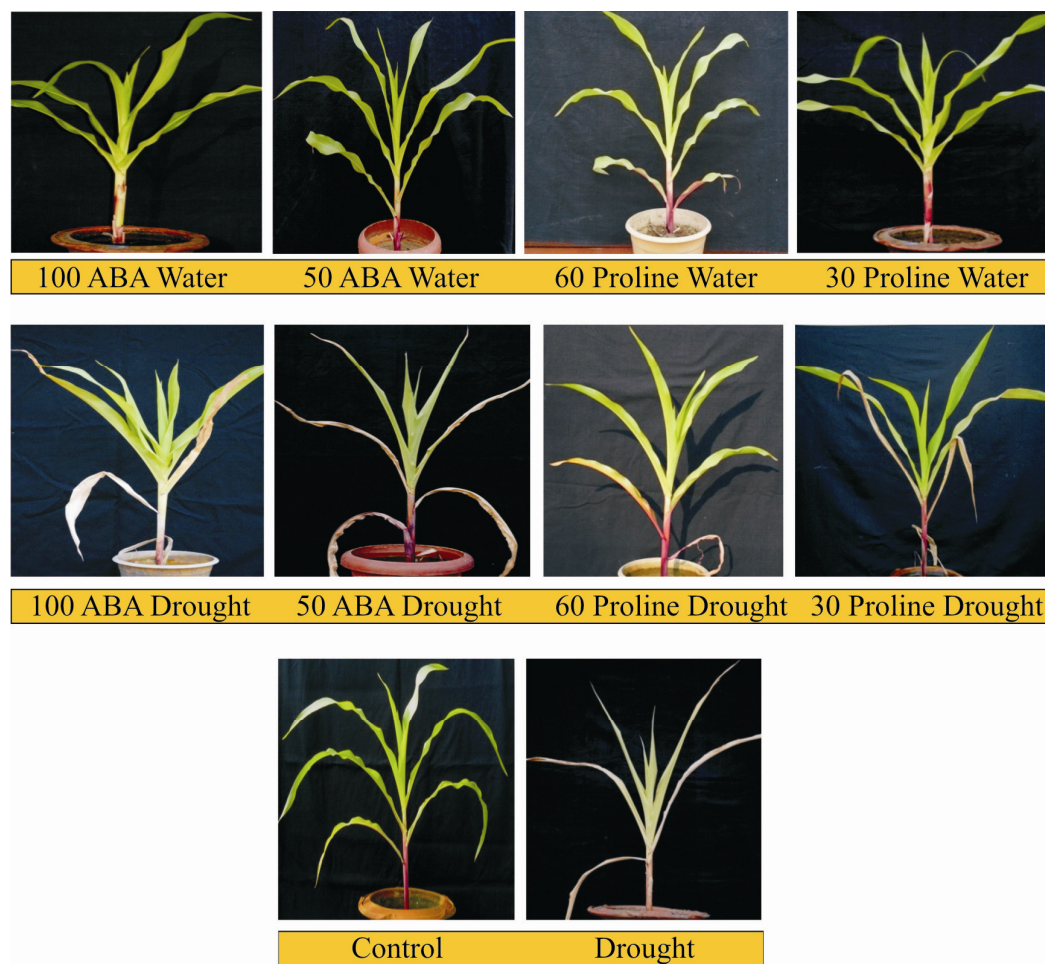
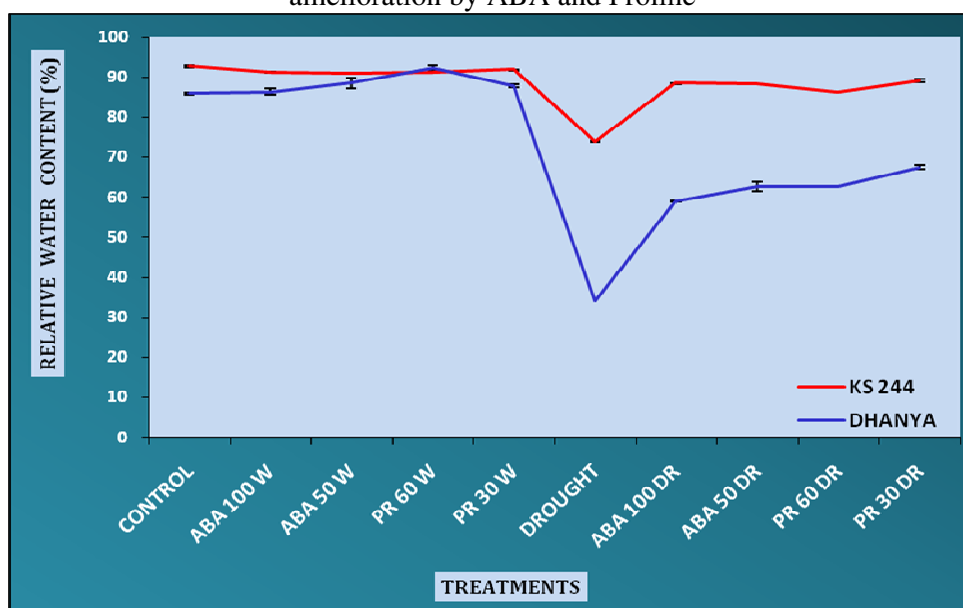


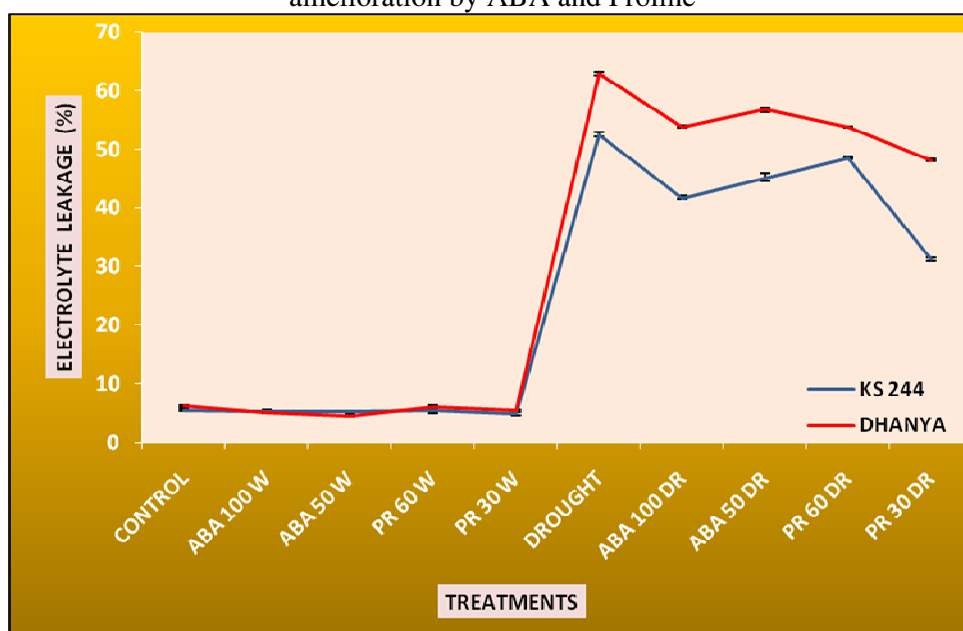
Fig 42: Amelioration of KS 244 cultivar by two chemicals abscisic acid (100 μ M and 50 μ M) and proline (60mM and 30mM)

Fig 43: Relative water content in leaves of two maize cultivars under water stress and amelioration by ABA and Proline



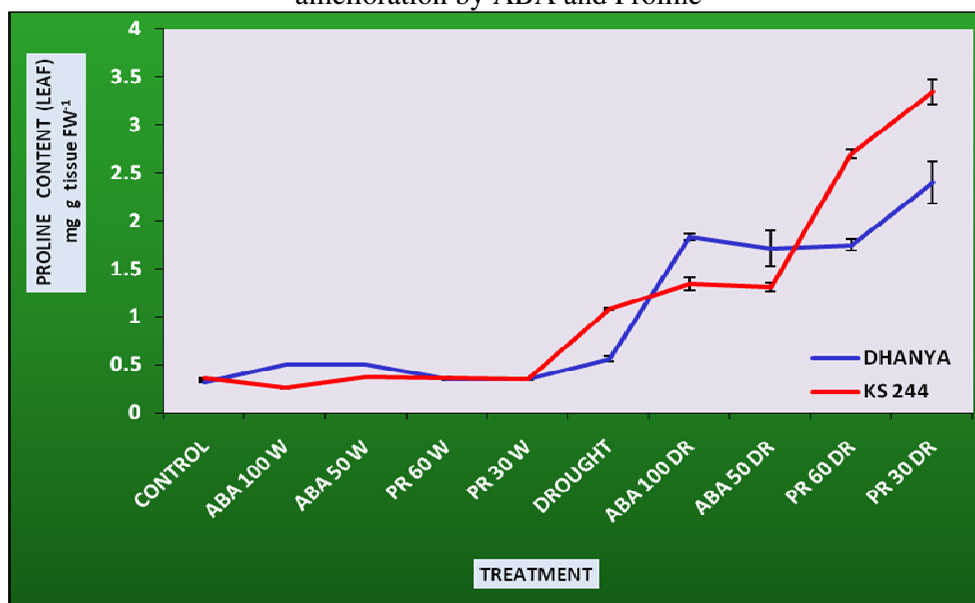
Different letters above different bars indicate the significant differences at $P \leq 0.010$ Students 't' test within each cultivar

Fig 44: Electrolyte leakage in leaves of two maize cultivars under water stress and amelioration by ABA and Proline



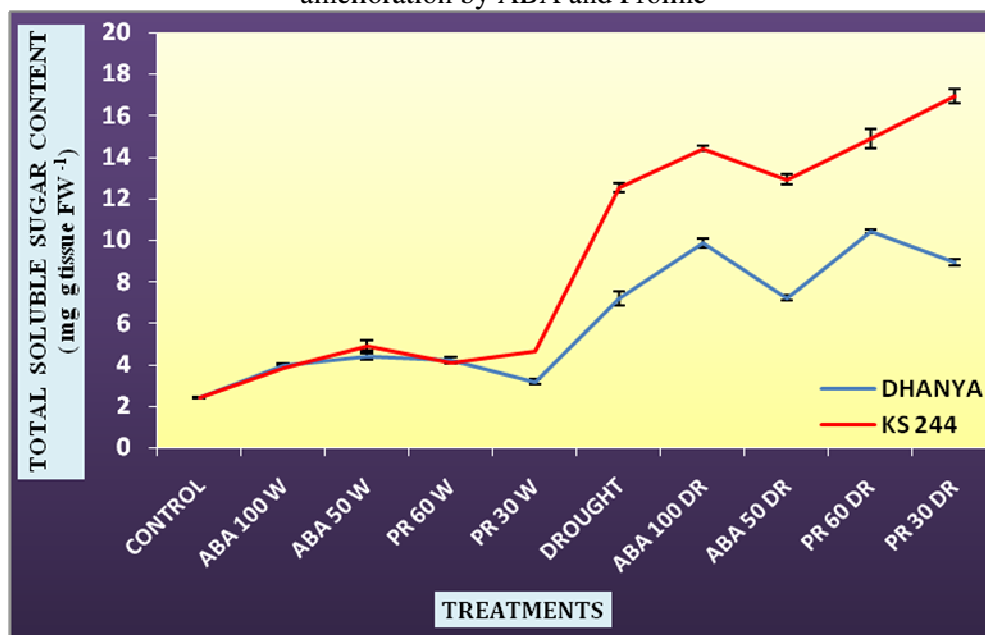
Different letters above different bars indicate the significant differences at $P \leq 0.010$ Students 't' test within each cultivar

Fig 45: Proline content in leaves of two maize cultivars under water stress and amelioration by ABA and Proline



Different letters above different bars indicate the significant differences at $P \leq 0.010$ Students 't' test within each cultivar

Fig 46: Total soluble sugar in leaves of two maize cultivars under water stress and amelioration by ABA and Proline



Different letters above different bars indicate the significant differences at $P \leq 0.010$ Students 't' test within each cultivar

4.16.6. Hydrogen peroxide accumulation

H₂O₂ accumulation increased in the leaves of both the cultivars during drought stress in comparison to the control. Much higher level of H₂O₂ was seen to accumulate in the susceptible cultivar (18 $\mu\text{mol g FW tissue}^{-1}$) during drought than in the tolerant cultivar (8.22 $\mu\text{mol g FW tissue}^{-1}$). Pre-treatment of both cultivars with ABA and proline revealed a decrease in H₂O₂ accumulation during drought in both cultivars. Though there was a decrease in all the pre-treated plants, maximum decrease was seen in plants treated with 30mM of proline. In case of Dhanya the level of H₂O₂ decreased from 18 to 8.74 in the 30mM treated plant which was highly significant (Table 42).

4.16.7. Antioxidative enzymes and antioxidants

Imposition of water stress in the leaves of the test cultivars showed marked changes in the activity of antioxidative enzyme like POX, APOX, CAT, SOD and GR. In the tolerant KS 244 cultivar, drought stress led to an increase in the POX and CAT activity (Table 41) but in case of the susceptible cultivar, drought stress led to a decrease in both the enzymatic activity. Plants treated with ABA and proline showed greater increase in the POX and CAT activity in KS 244 and also enhanced the activity in the susceptible cultivar. APOX (Table 41) and GR (Table 40) activity increased in plants subjected to drought stress in comparison to well watered control in both cultivars but the increase was very much significant in KS 244 than in Dhanya. Pre-treated plants showed further increase in the activity in both cultivars in all the four treatment. SOD activity decreased in both the cultivars in drought stress (Table 40), but plants pre-treated with ABA and proline enhanced the SOD activity in both the cultivars.

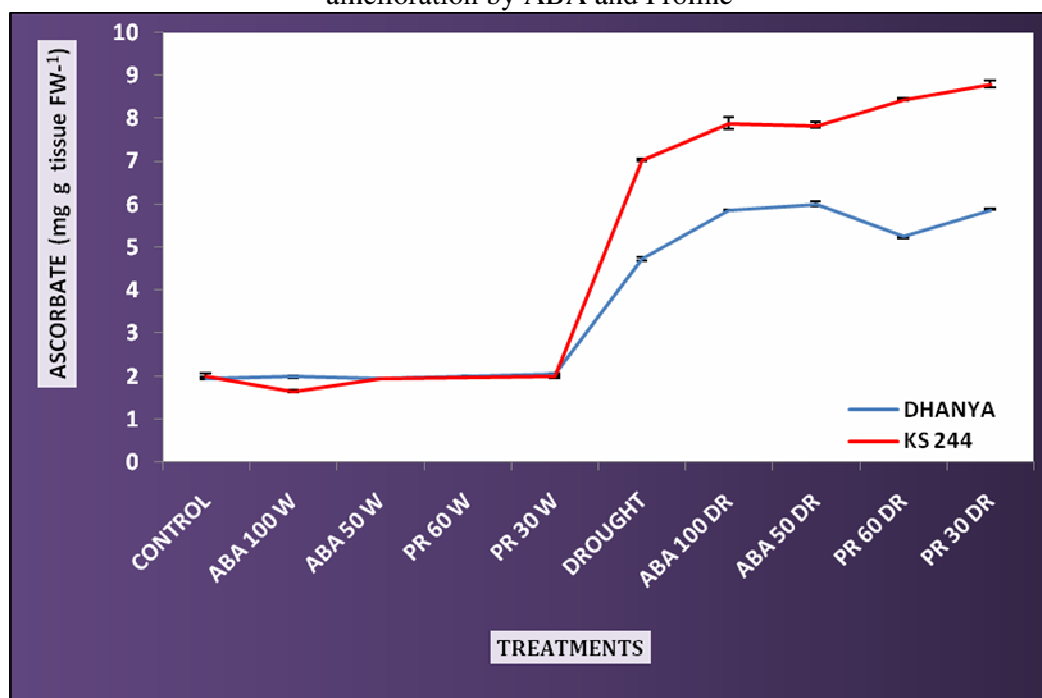
Enhanced accumulation of ascorbate was observed in the plants during drought stress. The tolerant cultivar KS 244 showed maximum increase in ascorbate content during drought stress. Pre-treatment of plants with ABA and proline further increased the ascorbate content in both cultivars under progressive drought. Enhanced accumulation of ascorbate was observed in the plants during drought stress. The tolerant cultivar KS 244 showed maximum increase in ascorbate content during drought stress. Pre-treatment of plants with ABA and proline further increased the ascorbate content in both cultivars under progressive drought (Fig 47).

Table 40: Effects of drought stress on superoxide dismutase and glutathione reductase activity in leaves of two maize cultivars and ameliorative effect of ABA and proline

TREATMENT	SOD		GR	
	KS 244	DHANYA	KS 244	DHANYA
CONTROL	0.53±0.0190 ^a	0.53±0.020 ^a	0.35±0.051 ^a	0.32±0.062 ^a
ABA100 W	0.51±0.003 ^a	0.52±0.003 ^a	0.34±0.034 ^a	0.36±0.036 ^a
ABA 50 W	0.57±0.006 ^a	0.48±0.010 ^a	0.35±0.035 ^a	0.32±0.062 ^a
PROLINE 60 W	0.63±0.033 ^a	0.49±0.007 ^a	0.42±0.052 ^a	0.35±0.044 ^a
PROLINE 30 W	0.62±0.033 ^a	0.51±0.003 ^a	0.39±0.038 ^a	0.34±0.049 ^a
DROUGHT	0.43±0.006 ^b	0.37±0.003 ^b	0.47±0.042 ^b	0.33±0.025 ^b
ABA 100 DR	0.54±0.006 ^a	0.49±0.009 ^a	1.08±0.030 ^b	0.58±0.022 ^b
ABA 50 DR	0.52±0.006 ^a	0.43±0.006 ^a	1.18±0.060 ^b	0.68±0.024 ^b
PROLINE 60 DR	0.56±0.012 ^a	0.65±0.009 ^b	1.21±0.021 ^b	0.58±0.022 ^b
PROLINE 30 DR	0.57±0.017 ^a	0.54±0.003 ^a	0.83±0.220 ^b	0.62±0.022 ^b

Results are expressed as mean of three replicates. Different superscript indicate significant differences in respect to control ($P < 0.010$), $\pm$ = Standard Error. Enzyme activities are expressed as SOD: EU mg protein⁻¹, GR: μ moles NADPH oxidized mg protein⁻¹ min⁻¹.

Fig 47: Ascorbate content in leaves of two maize cultivars under water stress and amelioration by ABA and Proline



Different letters above different bars indicate the significant differences at $P \leq 0.01$ Students 't' test within each cultivar

Table 41: Effect of drought stress on peroxidase, ascorbate peroxidase and catalase activity in leaves of two maize cultivars and ameliorative effect of ABA and proline

TREATMENT	POX		APOX		CAT	
	KS 244	DHANYA	KS 244	DHANYA	KS 244	DHANYA
CONTROL	0.98± 0.27 ^a	0.89± 0.044 ^a	0.31± 0.056 ^a	0.22± 0.062 ^a	0.47± 0.27 ^a	0.44± 0.111 ^a
ABA 100 W	0.93± 0.011 ^a	0.81± 0.055 ^a	0.21± 0.006 ^a	0.22± 0.036 ^a	0.42± 0.106 ^a	0.44± 0.110 ^a
ABA 50 W	0.92± 0.044 ^a	0.67± 0.019 ^a	0.22± 0.011 ^a	0.22± 0.062 ^a	0.44± 0.111 ^a	0.44± 0.111 ^a
PROLINE 60 W	0.94± 0.016 ^a	0.82± 0.024 ^a	0.32± 0.062 ^a	0.27± 0.044 ^a	0.32± 0.162 ^a	0.54± 0.137 ^a
PROLINE 30 W	0.80± 0.077 ^a	0.89± 0.031 ^a	0.35± 0.043 ^a	0.030± 0.049 ^a	0.47± 0.117 ^a	0.45± 0.260 ^a
DROUGHT	5.42± 0.111 ^b	0.76± 0.336 ^b	0.90± 0.065 ^b	0.32± 0.025 ^a	1.10± 0.065 ^a	0.16± 0.079 ^a
ABA 100 DR	6.60± 0.199 ^b	3.19± 0.039 ^b	0.99± 0.124 ^b	0.70± 0.022 ^b	1.24± 0.060 ^a	0.56± 0.070 ^a
ABA 50 DR	5.51± 0.210 ^b	3.45± 0.274 ^b	0.97± 0.069 ^b	0.60± 0.024 ^b	1.18 ±0.140 ^a	0.53± 0.075 ^a
PROLINE 60 DR	7.45± 0.07 ^b	4.09± 0.301 ^b	0.94± 0.067 ^b	0.70± 0.022 ^b	1.07± 0.067 ^a	0.56± 0.070 ^a
PROLINE 30 DR	6.63± 0.224 ^b	4.44± 0.127 ^b	1.07± 0.063 ^b	0.75± 0.022 ^b	1.26± 0.063 ^a	0.55± 0.068 ^a

Results are expressed as mean of three replicates. Different superscript indicate significant differences in respect to control ($P < 0.010$), $\pm$ = Standard error. Enzyme activities are expressed as POX: mmole O-dianisidine mg protein⁻¹ min⁻¹, APOX: m mole ascorbate mg protein⁻¹ min⁻¹, CAT: μ mole H₂O₂ mg protein⁻¹ min⁻¹.

Table 42: Drought stress induced changes in H₂O₂ accumulation, lipid peroxidation with pre-treatment of ABA and proline in maize leaves

TREATMENT	H ₂ O ₂ accumulation (μ M/g tissue FW)		Lipid peroxidation (μ M MDA/ g tissue)	
	KS 244	DHANYA	KS 244	DHANYA
CONTROL	3.60 $\pm$ 0.579 ^a	3.94 $\pm$ 0.150 ^a	0.005 $\pm$ 0.001 ^a	0.005 $\pm$ 0.0003 ^a
ABA100 W	4.80 $\pm$ 0.345 ^a	4.46 $\pm$ 0.204 ^a	0.008 $\pm$ 0.001 ^b	0.004 $\pm$ 0.0003 ^b
ABA 50 W	4.98 $\pm$ 0.863 ^a	5.48 $\pm$ 0.591 ^a	0.007 $\pm$ 0.0003 ^a	0.005 $\pm$ 0.0003 ^b
PROLINE 60 W	3.60 $\pm$ 0.303 ^a	4.46 $\pm$ 0.207 ^a	0.007 $\pm$ 0.0003 ^a	0.006 $\pm$ 0.0003 ^a
PROLINE 30 W	4.46 $\pm$ 0.207 ^a	4.80 $\pm$ 0.350 ^a	0.005 $\pm$ 0.0003 ^a	0.003 $\pm$ 0.0003 ^b
DROUGHT	8.22 $\pm$ 0.173 ^b	18.05 $\pm$ 1.319 ^b	0.012 $\pm$ 0.0003 ^b	0.031 $\pm$ 0.0015 ^b
ABA 100 DR	6.52 $\pm$ 0.199 ^b	14.22 $\pm$ 0.318 ^b	0.009 $\pm$ 0.0003 ^b	0.021 $\pm$ 0.0016 ^b
ABA 50 DR	5.83 $\pm$ 0.345 ^a	12.63 $\pm$ 0.204 ^b	0.010 $\pm$ 0.0001 ^b	0.018 $\pm$ 0.0018 ^b
PROLINE 60 DR	5.48 $\pm$ 0.595 ^a	10.12 $\pm$ 0.956 ^b	0.011 $\pm$ 0.0003 ^b	0.020 $\pm$ 0.0018 ^b
PROLINE 30 DR	6.18 $\pm$ 0.597 ^a	8.74 $\pm$ 0.098 ^b	0.009 $\pm$ 0.0003 ^b	0.017 $\pm$ 0.0003 ^b

Results are expressed as mean of three replicates. Different superscript indicate significant differences in respect to control ($P < 0.01$), $\pm$ = Standard error

Thus amelioration of drought stress *in vivo* in two cultivars of maize-KS 244 and Dhanya by two chemicals ABA (50 μ M and 100 μ M) and Proline (30mmol and 60mmol) showed that foliar application of ABA and proline was quite effective in ameliorating the adverse effects of water stress in maize by enhancing defense mechanisms, and the best results were seen in case of 30mmol proline application.

World population is expected to reach about (7.6-8.0) billion by the end of this century. Agricultural productivity is not increasing at a required rate to keep up with the global food demand, possible water shortages, depleting soil fertility and the various stresses constitute this handicap (Shanker and Venkateswarlu 2011). The growth and development of plants are severely restricted by a cultivar of environmental stresses such as drought, temperature, salinity, air pollution, heavy metals, pesticides and soil pH which are major limiting factors in crop production because they affect almost all plant functions (Sayfzadeh and Majid Rashidi 2010). Although most of the biochemical factors for stress tolerance acquisition are present in all species, the difference is how fast and how persistent this machinery is activated, and how the stress is perceived and how the signals are further transduced into a series of responses (Kaur and Gupta 2005). Among all the stresses, water stress is perhaps the most important since water is a vital component of all cellular processes (Shao *et al.* 2008). All plants have tolerance to water stress, but the extent varies from species to species. Knowledge of the biochemical and molecular responses to these stresses is essential for a holistic perception of plant resistance mechanisms to water stress in plants (Seyed *et al.* 2012).

In the present study the responses of seven cultivars of maize (*Zea mays* L.) to two kinds of water stress-drought and flooding was determined. These tested cultivars showed various degree of tolerance to drought and flooding stress. The seven cultivars of maize showed no morphological symptoms of drought stress on the 3rd day of stress but on the 5th day slight wilting was observed in all the seven cultivars. However after the 7th day severe wilting was observed in Dhanya, Swarna and BN 1133 which was much more pronounced than in KS 244, KS 244+, BN 101 and 30V92.

Tolerance index in the leaves of the seven test cultivars of maize during drought stress in pot culture showed that among the seven cultivars BN 101, KS 244 and KS 244+ had the highest tolerance index values on the 7th day of stress whereas Dhanya, Swarna, BN 1133 and 30V92 had very low tolerance level during the period of drought stress almost 30% less than the above three cultivars. This gives us the first line of indication that among the seven cultivars of maize KS 244, KS 244+ and BN

101 are more tolerant to drought stress. Similar results were seen in experiment performed by Raza *et al.* (2012) in wheat cultivars where significantly highest germination stress tolerance index was recorded in Lasani-2008 (97%), followed by AS-2002 (94%), which was at par with FSD-2008 (93%) and Pasban-90 (93%) who concluded that Lasani-2008 performed better under drought and Auqab-2000 was recorded as most drought sensitive cultivar. In experiments performed by Anwar *et al.* (2011) on 19 exotic genotypes of wheat, different drought tolerance indices were calculated on the basis of grain yield of the genotypes under irrigated (Yp) and stressed (Ys) conditions and it was depicted that greater the value of Tolerance index larger the yield reduction under stress conditions and higher the drought sensitivity. Negative value of Tolerance index showed more yield in stress than irrigated conditions.

Relative water content (RWC) which is an important marker of drought stress, is an integrative index of plant water status which is used to evaluate the tolerance to water stress (Gindaba *et al.* 2004). RWC in the leaves of the seven cultivars of maize in the present study was seen to decrease with the increase in number of days of drought stress in both *in vitro* and *in vivo* conditions in all the seven cultivars. RWC was significantly lower in Dhanya, Swarna and BN 1133 than in KS244, KS244+, BN101 and 30V92 during drought stress. Experiments performed on wheat by Arjenaki *et al.* (2012) also showed that drought on genotypes significantly ($p < 0.01$) affected RLWC. Kavir and Sardari genotypes which were the tolerant cultivars had the highest values of 79.96, 74.43%, respectively. Ghods genotype the susceptible cultivar had the lowest RWC of 59.3% during drought stress. Decrease in RWC in plants under drought stress may depend on plant vigor reduction and have been observed in many plants (Liu *et al.* 2002). Various studies have confirmed that the ability to maintain higher RWC is one of the mechanisms of drought tolerance in plants-Yang and Miao (2010) on two poplar species, Ali *et al.* (2010) in *Kochia (Kochia scoparia)* under saline condition, Parida *et al.* (2007) in the seeds of two genotypes of cotton, Chakraborty and Pradhan (2012) on wheat cultivars.

Another important marker of drought stress is electrolyte leakage of the test plant. The ability of cell membranes to control the rate of ion movement in and out of the cells is used as a test of damage to a great range of tissues (Valentovič *et al.* 2006). Cell membrane is one of the first targets of plant stresses (Levitt, 1972) and the ability of

plants to maintain membrane integrity under drought is what determines tolerance towards drought (Vieira da Silva *et al.*, 1974). Studies revealed that the leaves of the test plants showed greater electrolyte leakage during drought stress in all the seven cultivars of maize. *In vitro* drought stress showed that among them electrolytic leakage in Dhanya, Swarna, BN 1133 and 30V92 was 61.04%, 62.34%, 51.09% and 47.25% on the 7th day of stress whereas in BN 101, KS 244 and KS 244+ electrolyte leakage on the 7th day of stress was 24.96%, 30.54% and 27.74%. Thus, Dhanya, Swarna and BN 1133 showed greater electrolyte leakage during drought than the other four cultivars indicating that water stress-induced greater oxidative injury to these cultivars, and thus they are more sensitive to drought stress. The results from electrolyte leakage measurements showed that membrane integrity was conserved for tolerant compared to susceptible cultivars, this is in agreement with the conclusion of Martin *et al.* (1987) (six woody angiosperms) and Vasques-Tello *et al.* (1990) (*Phaseolous* and *Vigna*) Abdullah *et al.* (2011) (Syrian Durum Wheat) that electrolyte leakage was correlated with drought tolerance. The leakage is due to damage to cell membranes which become more permeable (Senaratana and Kersie, 1983). This shows the importance of this test in discriminating among tolerant and susceptible cultivars.

Membrane lipid peroxidation can lead to the destruction of cellular integrity, cell dysfunction and ultimately survival of plants (Ali *et al.* 2008). A variation in results was observed in lipid peroxidation in the leaves of seven cultivars during drought stress *in vitro* and *in vivo*. In Dhanya, Swarna and BN 1133 and 30V92 there was a significant increase in lipid peroxidation during prolonged drought stress whereas in the other three cultivars- BN 101, KS 244 and KS 244+ the lipid peroxidation did not show a significant increase during drought stress. In Dhanya, Swarna and BN 1133 and 30V92, during *in vivo* drought stress, on 0-day of treatment, they showed 0.008, 0.011, 0.011 and 0.016 μM MDA/ g tissue of lipid peroxidation respectively and on the 7th day it increased to 0.046, 0.047, 0.036 and 0.44 μM MDA/ g tissue respectively but KS 244, KS 244+ and BN 101 did not show such significant increase like Dhanya, Swarna and BN 1133. The observed changes in the MDA content were consistent with previous results observed by Fu and Huang (2001); Terzi and Kadioglu (2006); Chugh *et al.* (2011) where the authors stated that an enhanced level of lipid peroxidation of grasses and maize under drought stress indicated oxidative

damage to plant indicating that lipid peroxidation may be a consequence of generation of reactive oxygen species (OH, O₂⁻, H₂O₂). Thus lipid peroxidation is an important indicator of tolerance of a plant to drought stress. Unchanged lipid peroxidation seems to be a characteristic of tolerant plants that cope with environmental stresses like drought (Moussa and Abdel-Aziz 2008); so the results obtained in the present study indicates that among the test cultivars KS 244, KS 244+ and BN 101 are the most tolerant cultivars to drought stress, and Dhanya and Swarna cultivars are the most sensitive to drought stress.

Plant increases the activity of some involved enzymes in removing reactive oxygen species, like peroxidase, through stimulation of gene expression to alleviate the adverse effects of oxidative stress caused by drought. This condition is more visible in the resistant lines. The stress-resistant lines having these traits reduce damaging effects of oxidative stress through degradation and inactivation of reactive oxygen species and because of more stability of cell membrane and chlorophyll under these conditions, access to high yields in this lines is probable due to sustaining photosynthetic capacity. In drought stress conditions, the imbalance between energy intake and consumption by photosynthetic organ causes the production of reactive oxygen species (ROS) and inability of the plant to control them, which eventually led to stress in the cell membranes and incidence of symptoms caused by oxidative damage. Antioxidant enzymes are considered as the fastest units that fight against reactive oxygen species (Sharifi *et al.* 2012). Under any kind of stress condition generation of ROS is a common phenomenon observed in most of the plants. These stress-generated ROS damage the macromolecules such as DNA, proteins and lipid structures (Foyer and Noctor 2003; Ashraf and Fooland 2007; Ashraf 2009). And thus to counteract these ROS produced during stressful environments, plants in their cells start producing some key antioxidant enzymes such as peroxidase, catalase, ascorbate peroxidase, superoxide dismutase, glutathione reductase and polyphenol oxidase (Mittler 2002; Ashraf 2009; Ozden *et al.* 2009). In the present study also, these different stress related antioxidative enzymes exhibited different trends in activities during drought stress in the leaves of the seven cultivars of maize. In case of *in vivo* drought stress, assay of five antioxidative enzymes in seven cultivars revealed that four of the enzymes- POX, APOX, SOD and GR increased in all cultivars in the initial stage- ie., 3 days of water stress. However CAT activity in four of the cultivars-

Dhanya, Swarna, BN 1133 and 30V92 did not show much increase even in the initial period of stress and decreased in the latter period of stress. In these cultivars, POX and GR activity declined on 7th day of drought stress though it had been enhanced during 5 days stress in all the seven cultivars. Activities of APOX and SOD showed an initial increase during drought stress but on prolonged drought all the tested cultivars showed a decrease in the activity. Results of the present study clearly indicate that though there is an initial increase in activities of all antioxidative enzymes, high activities of POX, GR and CAT were maintained during prolonged drought in three cultivars- KS 244, K 244+ and BN 101. APOX, SOD and GR, on the other hand, showed similar trend in all cultivars. In case of *in vitro* drought stress assay of antioxidative enzyme activities showed that the POX activity increased in all cultivars in early stages of stress. In Dhanya, Swarna and 30V92 activity decreased on the 7th day but BN 101 and KS 244 showed a different trend where there was a slight decrease in POX activity on the 5th day and again increases on the 7th day. Maximum decrease in POX activity was seen in Dhanya. APOX activity was seen to decrease on the 7th day of stress in all five cultivars of maize. CAT activity showed a dramatic decrease in activity during drought in case of Dhanya, Swarna and 30V92 when compared to control but unlike these cultivars KS 244 and BN101 showed a significant increase in CAT activity during drought. SOD activity showed an increase in activity on the 3rd day of stress but on the 5th and 7th day there was a decrease in SOD activity in all cultivars of maize except for Swarna where SOD activity again showed a slight increase on the 7th day of stress after a decrease on the 5th day. Maintaining increased levels of GR, POX and CAT during prolonged stress could be one of the mechanisms responsible for their tolerance. SOD, being the first enzyme in the antioxidant pathway, increases initially in the tolerant cultivars, and contributes to the initial accumulation of H₂O₂. However, with increase in periods of stress, activities of SOD decline even in tolerant cultivars and the H₂O₂ produced is metabolized by POX. The combined action of CAT and POX converts the toxic superoxide radical (O²⁻) and hydrogen peroxide (H₂O₂) to water and molecular oxygen (O₂), thus averting the cellular damage under unfavorable conditions like drought stress (Noctor *et al.* 2002; Azevedo Neto *et al.* 2008). SOD is the first enzyme involved in the antioxidative process (Rubio *et al.* 2002) it is a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen

peroxide (Corpas *et al.* 2006) and plays a key role in the reduction of oxidative damages (Zhang *et al.* 2005). Enhancement of GR activity in tolerant cultivars indicated that tolerant plants exhibit a more active ascorbate- glutathione cycle than the less tolerant cultivars. GR, which catalyses the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) is an important endogenous antioxidant (Mc Kersie and Lesham 1994). The activity of GR which was relatively high in drought-stressed plants might be able to increase the ratio of NADP⁺/NADPH, thereby ensuring availability of NADP⁺ to accept electrons from photosynthetic electron transport chain and to facilitate the regeneration of oxidized ascorbate (Noctor *et al.* 2002). Higher antioxidant system activity was also observed in the drought-tolerant cultivars of wheat and rice (Lascano *et al.* 2001; Guo *et al.* 2006). Maintaining a high level of antioxidative enzyme activities may contribute to drought tolerance by increasing the capacity of better protection mechanisms against oxidative damage (Sharma and Dubey 2005; Türkan *et al.* 2005).

H₂O₂ was seen to accumulate in the leaves of all the cultivars during drought stress. Maximum accumulation was seen in Dhanya, Swarna and BN 1333, which increased to almost 80-90 folds on the 7th day of stress. Not much increase was seen in KS 244, KS 244+ and BN 101 during drought stress, and on the 7th day the H₂O₂ accumulation decreased in these cultivars in case of drought stress *In vivo* condition. Similar results were seen in case of *in vitro* drought stress. Thus it is quite clear that in cultivars which maintained higher levels of CAT and POX during prolonged periods of stress, H₂O₂ degradation was greater. Yang and Miao (2010) also noted the increments of the MDA and H₂O₂ concentrations in the water-stressed cuttings were 88.9 and 99.7% in *P. cathayana*, respectively, whereas they were only 44% and 63.6% in *P. kangdingensis*. Seedlings of selected six genotypes of maize (*Zea mays* L.) taken for studies by Chug *et al.* (2011) differing in their drought sensitivity (LM5 and Parkash drought-tolerant and PMH2, JH3459, Paras and LM14 as drought-sensitive) also showed similar results. H₂O₂ is a strong oxidant produced mainly as a result of scavenging of superoxide radical, and its higher concentration is injurious to cells, resulting in a localized oxidative damage, lipid peroxidation, and disruption of metabolic function and losses of cellular integrity at sites where it accumulates (Menconi *et al.* 1995; Velikova *et al.* 2000).

Ascorbate is among the many antioxidants, which protects cells against oxidative stress and has the capacity to eliminate different ROS including singlet oxygen, superoxide and hydroxyl radicals (Foyer 2001). Tocopherols are involved in protecting lipids and the photosynthetic apparatus against oxidative damage together with the antioxidants ascorbate, glutathione, and the photosystem-associated xanthophyll cycle (Porfirova *et al.* 2002; Sattler *et al.* 2004; Foyer and Noctor 2005; Havaux *et al.* 2005; Kanwischer *et al.* 2005). In the present study there was a significant increase of ascorbic acid and tocopherol in the leaves of the seven cultivars during drought stress both *in vivo* and *in vitro*, among which maximum increase was seen in KS 244, KS 244+ and BN 101 relating that these cultivars have better capacity to combat drought stress by eliminating different ROS produced during stress. Similar results in ascorbic acid content were also seen in experiments performed by Chakraborty and Pradhan (2012) in five cultivars of wheat (*Triticum aestivum* L.) during drought stress. A close relationship between antioxidant activity and stress tolerance has been identified in several crops (Zhang *et al.* 2003; Zhang and Ervin 2004; Misra and Gupta 2006).

Carotenoids are one of the major non-enzymatic anti-oxidants that have a role in defense against water stress by scavenging of singlet oxygen and suppressing lipid peroxidation in all photosynthetic organisms. Carotenoids a lipid soluble antioxidant plays a multitude of functions in plant metabolism including oxidative stress tolerance (Gill & Tuteja 2010). During *in vitro* and *in vivo* drought stress-the leaves of 30V92, BN 1133, Dhanya and Swarna cultivars showed a significant decrease in carotenoid content but incase of BN101, KS244 and KS 244+ there was a slight increase in carotenoid content during the initial period of stress. With prolonged drought stress the carotenoid content decreased in these cultivars too, but the decrease was not as much as that of the other four cultivars.

Accumulation of proline is an important indicator of drought stress tolerance in bacteria, algae, and higher plants. This amino acid has been reported to play multiple physiological functions in plants subjected to drought, such as osmoregulation, a sink for energy and nitrogen, and a signal of senescence (Aspinall and Paleg 1981). Accumulation of proline in plants under stress is a result of the reciprocal regulation of two pathways: increased expression of proline synthetic enzymes and repressed activity of proline degradation. Free proline is believed to play a key role in

cytoplasmic tolerance in many species and, therefore, in the resistance of the whole plant to severe drought (Seyed *et al.* 2012). Proline, which is usually considered as an osmoprotectant, may also be involved in reducing oxidative damage by scavenging the free radicals (Vendruscolo *et al.* 2007; Tatar and Gevrek 2008). In our study proline accumulation was seen in all the seven cultivars during drought stress in comparison to the control. Three cultivars-KS 244, KS 244+ and BN 101 showed maximum proline accumulation than the rest of the cultivars, almost four times more than that of the rest of the cultivars indicating its tolerance level to drought stress. Proline content was found to be more in the shoots than in the roots of the plants subjected to drought stress which was similar to the results obtained by Naser *et al.* (2010) on Persian walnut. Experiments performed by El Sayed (1992) in *Capsicum annuutn* L, Moussa and Abdel-Aziz (2008) and Ko's,kerog'lu and Tuna (2010), Mafakheri *et al.* (2010) in chickpea, also showed similar results during drought stress. Thus the increase in proline contents may be an adaptive mechanism, and increased proline content could induce drought tolerance (Yamada *et al.* 2005). Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure` 2009).

In the present study phenolic content in drought stress showed a decrease in all the seven test cultivars of maize in both *in vivo* and *in vitro* conditions. Maximum decrease was observed in 30V92, BN 1133, Dhanya and Swarna cultivars which was almost double of that of BN 101, KS 244 and KS 244+. This decrease in phenolic contents may be due to the degradation of photosynthetic pigments under drought stress. Results are supported by a study in which when maize plants were exposed to drought stress, the level of phenolic compound i.e., ferulic acid was decreased significantly due to the break down of the chlorophylls and phenolic pigments with the increase in drought intensity (Hura *et al.* 2007). Drought conditions reduced phenolic contents in cotton leaves at boll formation due to increased leaf senescence and degeneration of photosynthetic pigments (Ahmed *et al.* 2008).

It was also observed that during drought stress the sugar content in both leaves and root of all seven cultivars of maize increased in both *in vitro* and *in vivo* conditions but the raised soluble sugar fraction was accompanied by a sharp decrease in the starch fraction during drought in all the cultivars of maize. The tolerance mechanism

in water-deficit may be associated with accumulation of osmo-protectants such as proline and soluble sugars. The accumulation of total soluble sugars is strongly correlated to the acquisition of drought tolerance in plants (Hoekstra and Buitink 2001). Soluble sugars may be one of the main osmolytes in maize plants. They are used for osmotic adjustment. Fischer and Holl (1991) said that the increase in sugar concentration may be a result from starch degradation. In experiments performed by Mohammad khani and Heidari (2008) in two cultivars of maize, the concentration of soluble sugars increased, and at the same time a decrease in the starch concentration was observed in both cultivars which were very much similar to our result. Starch may play an important role in accumulation of soluble sugars in cells. Starch depletion in grapevine leaves was also noted by Patakas and Noitsakis (2001). Sugar content in leaves of Zayande-Rood cultivar of rice was higher than 829 and 216 cultivars in experiments performed by Mostajeran and Rahimi-Eichi (2009), the accumulation rate was correlated with drought tolerance. Thus in response to drought stress thus soluble sugars may also function as a typical osmo protectant, stabilizing cellular membranes and maintaining turgor pressure, thus free proline and soluble sugars can be used a drought tolerance indicator for selecting drought tolerant genotypes (Mostajeran and Rahimi-Eichi 2009). The maintenance of soluble sugar content may be achieved at the expense of starch, which drastically declines (Chaves 1991).

Drought is one of the factors affecting photosynthesis and chlorophyll content. Some of the researchers reported that chlorophyll content and chlorophyll fluorescence parameters might estimate influence of environmental stress on growth because these parameters were loosely correlated with the rate of carbon exchange (Araus *et al.* 1998; Guo and Li 2000; Fracheboud 2004) Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Both the chlorophyll a and b are prone to soil dehydration (Mohammadkhani and Heidari 2008; Farooq *et al.* 2009). In the present study the chlorophyll content was seen to decrease in the test plants subjected to

drought stress in both *in vivo* and *in vitro* conditions. Cultivars like Dhanya, Swarna, 30V92 and BN 1133, showed a marked decrease in chlorophyll content with prolonged drought stress, but the decrease in chlorophyll content was much lesser, in the rest of the four cultivars KS 244, KS 244+ and BN 101. Similar results have been given by Takele (2010), Efeoglu *et al.* (2009) in maize and Keyvan (2010) in bread wheat.

Total antioxidant activity by DPPH inhibition of seven cultivars of maize showed a decrease in the activity during drought stress, in both *in vivo* and *in vitro* conditions. In *in vitro* drought stress, maximum decrease was seen in case of BN 1133, Dhanya and Swarna during prolonged drought stress, where DPPH inhibition on the 7th day of stress was 45.63%, 48.58% and 50.81 % respectively, where as in KS 244, KS 244+ BN 101 showed an increase during period of drought when compared to the control. Similar results were seen in case of *in vivo* drought stress too. Similar results were seen in experiments performed by Chakraborty and Pradhan, (2012) in five cultivars of wheat (*Triticum aestivum* L.) during drought stress, in experiments performed by Ali *et al.* (2010), in maize where there was decrease in DPPH scavenging activity due to water stress along with phenolics and carotenoid content, in experiments performed by Dykes and Rooney (2007) in cereal grain and Kumar *et al.* (2009) in soybean.

It is clear that the oxidative stress induced by drought stress in maize cultivars is countered by the over production of stress responsive metabolites, antioxidants and antioxidative enzymes, specially in the KS 244, KS 244+ and BN 101 cultivars which could withstand water stress more efficiently.

The results obtained from field trials done on KS 244 and Dhanya makes the above statement stronger that KS 244 is the tolerant cultivar and Dhanya is the susceptible cultivar among the test cultivars used for the study.

Flooding is a natural disturbance affecting crop and forage production worldwide due to the detrimental effects that it provokes on most terrestrial plants (Bailey-Serres & Voesenek 2008; Colmer & Voesenek 2009). Over the last years, the Intergovernmental Panel on Climate Change (<http://www.ipcc.ch>) has informed that man-induced world climate change will increase the frequency of precipitations of higher magnitude as well as tropical cyclone activity. As a result, the occurrence of flooding events on flood plains (i.e. lowlands) and cultivated lands is expected to be higher (Arnell and

Liu 2001). On the other hand, the increasing world population, along with the intensification of agriculture have provoked a reduction in the arable land per capita, which has decreased over the last five decades from 0.32 ha to 0.21 ha, and it is expected to be further diminished up to 0.16 ha per capita by 2030 (Mancuso and Shabala 2010). As a consequence, marginal lands are being incorporated into production to cope with the rising food demand. These issues lead to the necessity to get highly productive crops in arable lands subjected to periodic events of water excess, and to introduce new (or improved) flood-tolerant forage species in flood prone pastures (and grasslands) devoted to livestock production. So, the understanding of plant functioning under flooding conditions is crucial in order to achieve these goals (Striker 2012). Water logging usually results from overuse and/or poor management of irrigation water and is a serious constraint with damaging effects. The rapidly depleting oxygen from submerged root zone is sensed and plant adjusts expressing anaerobic proteins. Plant cells shift their metabolism towards low energy yielding anaerobic fermentation pathways in the absence of oxygen. Structural modifications are also induced as aerenchyma formation and adventitious rootings, etc. Studies at molecular and biochemical levels to facilitate early perception and subsequent responses have also been worked out to produce resistant transgenic plants (Irfan *et al.* 2010)

In the present study seven cultivars of maize-KS 244, KS 244+, BN 101, 30V92, BN 1133, Dhanya and Swarna, which were tested to determine drought stress, were also tested to determine their tolerance to flooding stress. Unlike that of drought stress, in flooding stress it was seen that the RWC in these cultivars did not show a significant decrease even during prolonged period of flooding. In KS 244, KS 244+ and BN 101 the reduction on the 7th day of stress was 4-5% and in 30V92, BN 1133, Dhanya and Swarna reduction was 7-8% in comparison to control plants. Similar results were seen in experiments performed by Jahan *et al.* (2013) in rice, Celik and Turhan (2011) in bean. This helps us to understand that flooding stress dose not have much effect RWC of the plants.

There was an increase in the electrolyte leakage and lipid peroxidation in the seven cultivars during flooding stress in comparison to the control plants. Maximum increase was seen in BN 1133, Dhanya and Swarna. Intensification of lipid peroxidation under soil flooding was shown both in the heterotrophic root tissues

subjected directly to the action of soil hypoxia and in the photosynthesizing organs surviving an indirect effect of this stress factor (Hunter *et al.* 1983; Yan *et al.* 1996; Zakrzhevsky *et al.* 1995 Balakhnina *et al.* 2004, 2009). The plant capability to activate the oxidative defense system is the main mechanism providing plant resistance against oxidative damage. This resistance is mainly connected with changing the activity levels of one or more antioxidant enzymes (Allen 1995). Lipid peroxidation, a measure of free radical generation, which is determined in terms of malondialdehyde (MDA) content (Jamwal *et al.* 2012). Malondialdehyde (MDA) is one of the end breakdown products of lipid peroxidation and can be used as an indicator of lipid peroxidation, it results from producing active oxygen types like superoxide radical, hydrogen peroxide and hydroxide radical and is an indicator of lipid peroxidation in plant tissue resulting from an oxidative stress (Xue and Liu 2008). Thus the electrolyte leakage and MDA accumulation results indicate that among the seven cultivars of maize, the same cultivars that were tolerant to drought stress are also tolerant to flooding stress.

Excessive generation of reactive oxygen species (ROS), or oxidative stress, is an integral part of many stress situations, including hypoxia Biemelt *et al.* (2000). Protection of plants from this oxidative damage is associated with functioning of low molecular antioxidants (carotenoids, ascorbic acid, glutathione etc.) and various antioxidant enzymes such as superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, glutathione reductase, the enzymes of ascorbate-glutathione cycle and other pathways neutralizing ROS (Asada 2006). In our present study also, the seven cultivars of maize show different trends in the activity of various stress related antioxidative during flooding stress. POX, APOX and SOD activity in KS 244, Ks 244+ and BN 101 increased till the 5th day of stress and decreased on the 7th day of stress but in 30V92, BN 1133, Dhanya and Swarna the activity decreased from the 5th day of stress, there was only an initial increase on the 3rd day of stress. But CAT and GR activity showed a gradual and significant increase in KS 244, KS 244+ and BN 101 during flooding stress. In other cultivars like in drought stress, and opposite trend was seen where the CAT activity decreased from the initial period of stress and GR activity showed an initial increase and then decreased in the activity from the 5th day of stress. Similar results were seen where the activity of GR in *Vicia faba* leaves decreased already after 2 days of soil hypoxia (Balakhnina *et al.* 2009.) and in

experiments performed by Qiang *et al.* (2011) in Jerusalem artichoke. According to (Biemelt *et al.* 1998) the wheat seedlings after 8 days of hypoxic stress development showed a decrease in the GR activity in the roots. Authors explained these results as an acclimatization of wheat roots to hypoxia. The analogous decrease in the GR activity was also found in the maize leaves (Yan *et al.* 1996) after 7 days of soil hypoxia as compared to the controls.

Hydrogen peroxide in the seven cultivars of maize was seen to accumulate more during flooding stress. Maximum accumulation was seen in BN 1133, Dhanya and Swarna which was almost 30-50% more than in the 0-day of treatment. Hydrogen peroxide accumulation under hypoxic conditions has been shown in the roots and leaves of *Hordeum vulgare* (Kalashnikov *et al.* 1994) and in wheat roots (Biemelt *et al.* 2000). The presence of H_2O_2 in the apoplast and in association with the plasma membrane has been visualized by transmission electron microscopy under hypoxic conditions in four plant species (Blokhina *et al.* 2001).

Protection of plants from oxidative damage is associated with functioning of low molecular antioxidants -carotenoids, ascorbic acid, glutathione etc (Asada 2006; Elstner 1982; Salin 1988). Antioxidants like ascorbate and tocopherol also increased during flooding stress in all the seven cultivars like in drought stress, and maximum accumulation was seen in case of KS 244, KS 244+ and BN 101. The carotenoid content showed a decrease during flooding stress with maximum decrease in BN 1133, Dhanya and Swarna. Similar results were also observed in experiments performed by Tamara *et al.* (2010), in *Vicia fabia*. Even in experiments performed by Balakhnina *et al.* (2009) in *Vicia faba* carotenoid concentration in the leaves of flooded plants, as compared with the control ones, decreased significantly after 15 days of soil flooding.

One of the most common stress responses in plants is over production of different types of compatible solutes such as proline and betaine (Serraj and Sinclair 2002) which are low molecular weight highly soluble compounds that are usually non-toxic at high cellular concentration. Generally they protect plants from stress through various mechanisms including contribution to cellular osmotic adjustment, detoxification of ROS, protection of membrane integrity and stabilization of enzymes or protein (Ashraf and Fooland 2007). Proline content in the seven cultivars increased

in both the roots and the leaves during flooding stress, but in BN 1133, 30V92, Dhanya and Swarna there was a significant decrease in proline content both in the leaves and root of these cultivars on the 7th day of flooding stress. Similar results were seen in case of total soluble sugar and reducing sugar of the seven cultivars of maize during flooding stress. The accumulation of soluble sugar in roots of *L. latifolium* suggests that the availability of soluble sugar is not limiting to metabolic activity in roots. The primary use of carbohydrate reserves is to maintain respiration and growth in the absence of photosynthesis (Hook and Brown 1973). The supply of soluble sugar allows roots to respire anaerobically under flooding conditions. The observation that an exogenous supply of glucose enhances survival of crop plants under flooding stress (Webb and Armstrong 1983) has confirmed this importance.

Chlorophyll content like in drought stress also showed a reduction during flooding stress in all the seven cultivars of maize and maximum decrease was seen in BN 1133, Dhanya and Swarna, confirming that these three cultivars are highly sensitive to flooding stress than the rest of the cultivars.

Results of the present study therefore indicate that flooding stress induces oxidative stress in all the seven cultivars of maize, but as antioxidative mechanism was much more pronounced in BN 101, KS 244 and KS 244+ these cultivars were the most tolerant to flooding stress.

Since there are earlier reports on ameliorative effects of certain chemicals during drought, in the present study experiments were conducted to determine whether ABA and proline had ameliorative effects against drought. One of the early symptoms of water deficiency in plant tissues is the decrease of relative water content. Drought stress led to a decrease in the RWC of leaves in both the cultivars where the tolerant cultivar KS 244 showed a lesser decrease than the susceptible cultivar Dhanya. This result was in conformity to that of Bano *et al.* (2012), where they showed that the drought tolerant cultivar showed lower decrease in the RWC than the susceptible cv. Punjab-96. Exogenous application of ABA and proline led to an increase in the Relative Water Content of both cultivars of maize than in non-treated plants subjected to drought stress. Drought stress led to an increase in electrolytic leakage in both cultivars, much more in the susceptible cultivar (Dhanya) than in the tolerant cultivar (KS 244). Amelioration with ABA and proline led to a decrease in the electrolytic

leakage, with 30mmol of proline being most effective. Proline has been studied extensively by many researchers all over the world. Along with its direct protective role as an osmolyte, it also improves water relations under drought conditions. Many other physiological roles have been assigned to proline, including stabilization of macromolecules, a sink for excess reductant, and a means of storing carbon and nitrogen for use after relief of water deficit (Wahid and Close 2007). In our finding, proline is seen to increase in the plants during drought stress, and the increase is much more in the tolerant cultivar KS 244 than in Dhanya, the susceptible cultivar. Thus the increase in proline contents may be an adaptive mechanism, and increased proline content could be either due to increased biosynthesis or decreased breakdown. Similar to proline, accumulation of ascorbate was also comparatively higher during drought stress. The tolerant cultivar exhibited more of these metabolites and antioxidant during drought stress and even greater in the pre-treated plants indicating a better protection mechanism in the plant. ROS generated during drought stress increases the contents of malondialdehyde (MDA), which is considered as a suitable marker for membrane lipid peroxidation. In our study the MDA content increased in both the tolerant and susceptible cultivar during drought stress, but greater MDA accumulation was seen in the susceptible cultivar. Amelioration of the two cultivars by ABA and proline led to a decrease in both the cultivars. Membrane lipid peroxidation can lead to the destruction of cellular integrity, cell dysfunction and ultimately survival of plants.

In order to cope with potentially cytotoxic forms of activated oxygen species, plants have evolved a battery of antioxidative enzymes, such as SOD, POX, APOX, GR and CAT as well antioxidants such as ascorbic acid, α -tocopherol and carotenoids which function as an extremely efficient cooperative system. In our studies pre-treatment of both the cultivars with foliar application of ABA and proline, there was an increase in all the antioxidative enzyme activity especially in the Dhanya cultivar during drought stress, where in the same cultivar untreated plants did not show such increase in the activity indicating that pre-treatment of the plants with these chemical led to better efficiency of the plant to cope with stress similar results was seen in experiment performed by Bano *et al.* (2012) in wheat where ABA treated plants in experiments showed a significant increase in POD activity under drought stress. Thus our results thus suggest that ABA and proline treated plants exhibit increased POX, CAT,

APOX, SOD and GR activities, indicating a more efficient quenching of ROS. Levels of H_2O_2 accumulation was seen to increase during drought stress in both KS 244 and Dhanya, but greater level of H_2O_2 was seen in Dhanya cultivar. This is probably due to the inability of scavenging enzymes POX and CAT to breakdown H_2O_2 in the susceptible cultivar. Amelioration by ABA and proline led to a decrease in H_2O_2 level in both the cultivars. ABA was reported to significantly enhance the antioxidant enzymes activity in maize seedlings subjected to water stress (Jiang and Zhang 2002).

Obtained data thus indicate water stress induced oxidative injury, especially in drought-sensitive maize plants, as evidenced by increases in lipid peroxidation, H_2O_2 content and other ROS markers. Antioxidative mechanisms were more pronounced in one of the cultivars which were more tolerant to drought. Thus from the present study we can say that the foliar application of ABA and proline was effective in ameliorating the adverse effects of water stress in maize by enhancing defence mechanisms not only in KS 244-the tolerant cultivar but also in- Dhanya which was found to be very susceptible to drought stress.

1. A review of literature has been presented on previous work done in the line of investigation. The review has been presented on abiotic stress in plants giving special reference to drought stress and flooding stress in plants, and the metabolic changes associated with these stresses.
2. Experimental procedure and protocol used in this investigation have been presented in detail.
3. Seven cultivars of maize (*Zea mays* L.) have subjected to five sets of treatment in different stages-**A**: Two month old plants of all the seven test grown in the glass house was subjected to drought stress by completely withholding water in the pots of the test plants, and then the sampling of the plants were performed on the 3rd, 5th, and 7th day of drought stress along with the sampling of the well watered control plants. **B**: One week old seedlings of all the seven cultivars was subjected to drought stress by applying 10% (w/v) PEG-6000 (Polyethylene glycol), and sampling was done on the 3rd, 5th and 7th day of stress along with the sampling of the control plants which was done on 0-day. **C**: One set of two week old plant of all the seven cultivars of maize that was grown in thermocoil cups was subjected to flooding stress by holding water in the pots. The test plants were then sampled on the 3rd, 5th and 7th day of stress. Control plants were sampled on the 0-day of stress. **D**: One month old plants of two cultivars KS 244 and Dhanya in pot culture was given foliar spray of two chemicals ABA and proline of two different concentration each prior to drought stress and then various biochemical assays were performed on the treated and the untreated plant to see the ameliorating effects of the chemicals on the two cultivars **E**: One week old seedlings of two cultivars KS 244 and Dhanya (DMH 849) was transferred to a plot made in the field and was watered daily till the time of flowering, then the plants were subjected to drought stress by withholding water and drought subjected plants were sampled on the 20th day of drought stress along with the control which was sampled on 0-day of stress.
4. Tolerant and susceptible cultivars of maize were screened by performing cell membrane stability test, tolerance index and relative water content. Cultivars-KS 244, KS 244+ and BN 101 showed maximum cell membrane stability, maximum

tolerance index and very less decrease in relative water content during stress, which gave the first line of indication that these cultivars are the most tolerant cultivars among the seven test cultivars of maize and since BN 1133, Swarna and Dhanya showed minimum cell membrane stability, minimum tolerance index and a significant decrease in relative water content during water stress showed that these cultivars are the most susceptible cultivars during water stress.

5. Lipid peroxidation was determined as the amount of malondialdehyde (MDA) in the membrane. In the present study- cultivars BN 1133, Dhanya and Swarna was seen to have maximum MDA accumulation during water stress, indicating that these cultivars are very much sensitive to water stress.
6. Protein content increased during water stress in both the leaves and the roots of KS 244, KS 244+ and BN 101. But in BN 1133, Swarna and Dhanya the protein content decreased in prolonged drought stress in both the leaves and the root of these cultivars.
7. The assay of antioxidative enzymes in the seven cultivars of maize showed varying trends in their activity in different types of stress.
 - A. *In vitro* drought stress POX activity in KS 244, KS244+ and BN101 showed a slight decrease in activity on the 3rd day of drought stress, but again the activity increased on the 7th day of stress. In the other cultivars the activity declined drastically on the 7th day of stress. CAT and GR activity showed a similar trend in KS 244, KS 244+ and BN 101 where the activity showed a significant increase in the activity during drought stress. But in case of 30V92, BN 1133, Dhanya and Swarna a steep decline in the activity was observed during the period of stress. APOX and SOD activity in all the seven cultivars increased in the initial stages of stress but during prolonged stress a decrease in the activity of these antioxidative enzymes was observed
 - B: POX activity in the seven cultivars in case of *in vivo* drought stress showed different trend in different cultivars. In KS 244+, KS 244 and BN 101 POX activity increased significantly with drought stress. But in BN 1133, 30V92, Swarna and Dhanya the activity decreased on the 7th day of stress. APOX activity increased in the initial period of stress and then decreased after the 7th day in all the cultivars. Similar type of trend was seen in case of SOD, where the

activity decreased after the 5th day of stress in all the seven cultivars. GR and CAT activity in KS 244, KS 244+ and BN101 increased with the increase in drought stress but in the other four cultivars i.e BN 1133, 30V92, Swarna and Dhanya the GR and CAT activity showed very insignificant increase during the initial stages of drought with a decrease in the later stage of stress.

- C:** During flooding stress antioxidative enzymes like POX, CAT, APOX, SOD and GR showed different trends in their activity in different cultivars. POX activity showed an increasing trend in the activity in the initial days of flooding stress but gradually with prolonged stress the activity decreased in all the test cultivars. In KS 244, KS 244+ and BN 101 the activity decreased on the 7th day of stress and in BN1133, 30V92, Swarna and Dhanya the activity decreased from the 5th day of stress. CAT activity showed a gradual, significant increase in KS 244, KS 244+ and BN 101. But in BN 1133, 30V92, Swarna and Dhanya the catalase activity decreased during the period of stress. APOX activity and SOD activity in BN 101, KS 244, KS 244+ showed an increase in activity till the 10th day of flooding stress but a decrease in the activity was seen on the 7th day of stress a different trend was seen in case of BN 1133, Swarna, Dhanya and 30V92 where the APOX activity showed an initial increase and then decreased in activity after the 3rd day stress. The GR activity in KS 244, KS 244+ and BN 101 showed a significant increase in activity during the period of flooding stress. But like APOX and SOD, the GR activity showed a same type of trend in BN 1133, Swarna, Dhanya and 30V92 during flooding stress.
8. Protein profile was analyzed by SDS-PAGE in the test cultivar. Expression of new protein bands were seen in the seven cultivars of maize during drought stress.
 9. Isozyme analysis was done by native PAGE. Peroxidase isozyme analysis revealed the presence of new bands in during water stress in most of the cultivars.
 10. Antioxidants like ascorbate and α -tocopherol was seen to increase during water stress in all the seven cultivars of maize. Over all a decrease in carotenoids were seen in all the seven cultivars of maize during water stress long with a decrease

in chlorophyll content also. Maximum decrease in the chlorophyll content was seen in BN 1133, Dhanya and Swarna during water stress.

11. Proline content and sugar content in both the leaves and the roots of the test plant increased during water stress. It was seen that proline content was greater in the leaves of the test plant than in the roots of the plant but unlike proline content the accumulation of sugar was greater in the roots than in the leaves of the plants during drought stress. But in 30V92, BN 1133, Dhanya and Swarna the sugar content showed a significant decrease on the 5th day of flooding stress. Unlike proline and sugar content, there was a decrease in the starch content in the seven cultivars of maize during water stress. The decrease was seen both in the leaves and the roots of the test plants.
12. The total antioxidant activity in the leaves of the test plants was determined with respect to percent of DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) inhibition. During *in vitro* drought stress and *in vivo* drought stress it was seen that in cultivar KS 244, KS 244+ and BN 101, there was an increase in the DPPH inhibition during the period of stress. In case of 30V92 a decrease was seen on the 7th day of stress and in BN 1133, Dhanya and Swarna there was a slight increase on the 3rd day of stress but a significant decrease was seen on the 5th and the 7th day of water stress. In flooding stress KS 244, KS 244+, 30V92 and BN 101 increased during the period of stress but showed a slight decrease in the DPPH inhibition on the 7th day of stress. The other cultivars showed a significant decrease in the DPPH inhibition during the period of stress.
13. Water stress in the maize cultivars resulted in the increase in hydrogen peroxide accumulation in the leaves of the test cultivars. Maximum H₂O₂ accumulation was seen in case of BN 1133, Dhanya and Swarna. In KS 244, Ks 244+ and BN 101 the accumulation of H₂O₂ was very negligible in comparison to the control plants.
14. During water stress a decrease in the phenolic content was observed in all the seven cultivars of maize. HPLC analysis of total phenol and phenolic acids of two cultivars of maize-KS 244 and Dhanya during *in vivo* drought and flooding stress showed that some of the peaks corresponding various total phenols and phenolic acid disappeared in the test plants subjected to water stress. There was

also a significant decrease in the height of the peaks in the plants subjected to stress in comparison to the control.

15. Amelioration of drought stress *in vivo* in two cultivars of maize-KS 244 and Dhanya by two chemicals ABA (50 μ M and 100 μ M) and proline (30mmol and 60mmol) showed that foliar application of ABA and proline was effective in ameliorating the adverse effects of water stress in maize by enhancing defense mechanisms and the best results were seen in case of 30mmol proline application.
 - i. An increase in RWC was seen in the pre-treated plants during drought stress. The best results were seen in case of the plant treated with 30mM of proline where RWC was 67.5% in the susceptible cultivar after drought stress, whereas the untreated plant under drought stress showed 34.23% of RWC.
 - ii. Plants treated with ABA and proline treatment prior to stress exhibited electrolyte leakage values less than the untreated plants subjected to drought stress. This difference was seen in case of both the cultivars. The mechanism of ABA induced improvement of electrolyte leakage appears to be mediated via increased accumulation of osmolytes and enhanced activities of antioxidants.
 - iii. Pre-treated plants showed an increase in the antioxidative enzyme activity in both cultivars in all the four treatment. SOD activity decreased in both the cultivars in drought stress, but plants pre-treated with ABA and proline enhanced the SOD activity in both the cultivars.
 - iv. Amelioration with ABA and proline resulted in the decrease of MDA content of both the test cultivars, where again plants pre-treated with 30mmol of proline showed best results.
 - v. Pre-treatment of both cultivars with ABA and proline revealed a decrease in H₂O₂ accumulation during drought in both cultivars. Though there was a decrease in all the pre-treated plants, maximum decrease was seen in plants treated with 30mM of proline. In case of Dhanya the level of H₂O₂ decreased from 18 to 8.74 in the 30mM treated plant which was highly significant.
 - vi. Pre-treatment of plants with ABA and proline further increased the ascorbate content in both cultivars under progressive drought.

- vii.** Pre-treatment of both cultivars resulted in greater accumulation of proline during drought and best results were seen in plants treated with 30mmol of proline. Both the cultivar showed almost three fold increases in the proline content in plants treated with 30mmol of proline than the untreated plant subjected to drought stress.
16. Results of all experiments revealed that out of the seven test cultivars of maize- KS 244, KS 244 and BN 101 are the more tolerant cultivars to both types of water stresses and BN 1133, Dhanya and Swarna are the susceptible cultivars to both type of water stresses. Relative water content, electrolyte leakage, lipid peroxidation, tolerance index, free proline, total soluble sugars and chlorophyll content were found to be very important markers of water stress.
17. Statistical analysis of data has been done by ANOVA and t-test to analyze the difference in between the treatments and the difference in between the seven test cultivars.

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APPENDICES

APPENDIX A: List of thesis related publications

1. **Chakraborty U and Lama R.** Evaluation of Antioxidant Responses of Maize (*Zea mays* L.) Varieties to Water Stress. *J. Plant Biol.* **37 (1):** 99–107, 2010.
2. **Lama R and Chakraborty U.** Polyethylene glycol induced water stress in maize seedlings and evaluation of antioxidant defense mechanisms. *NBU J. Plant Sciences.* **6(1):** 35-40, 2012.
3. **Lama R and Chakraborty U.** Water Deficit Stress Tolerance Traits in Maize (*Zea mays* L.) and Identification of Tolerant Varieties. *Int. J. Bio-resource and Stress Management.* **4(2)** special: 329-334, 2013.
4. **Allay S, Lama R, Chakraborty U and Chakraborty BN.** Antioxidative responses of mandarin plants to water stress. *NBU J. Plant Sciences.* **7(1):** 21-28, 2013.

In Proceeding volume:

Chakaraborty U, Pradhan D, Lama R, Pradhan B, De U and Chakaraborty BN. Abiotic stress induced biochemical response in cereals and legumes and associated changes in arbuscular mycorrhizal population. (Eds. Bishwanath Chakraborty and Usha Chakraborty), Satish Publishing House, 183-198, 2013.

APPENDIX B: List of Abbreviations

ABA- Absciscic acid
 APOX-Ascorbate peroxidase
 APS-Ammonium persulphate solution
 ASA- Ascorbic acid
 ASI -Anthesis silking interval
 Ca (NO₃)₂.4H₂O- Calcium nitrate tetrahydrate
 CAR-Carotenoid
 CAT- Catalase
 CO₂- Carbon dioxide
 Cu-Copper
 CuSO₄.5H₂O-Copper sulphate pentahydrate
 DAB -3, 3'-Diaminobenzidine
 DHAR- Dehydroascorbate reductase
 DNA- Deoxyribonucleic acid
 DPPH-1, 1-Diphenyl-2-picryl-hydrazyl
 DW-Dry weight
 EDTA- Ethylene diamine tetra acetic acid
 EL-Electrolyte leakage
 FC- Field capacity
 FeSO₄-Ferrous sulphate
 FW-Fresh weight
 g- Gram
 GB- Glycinebetain
 GOPX- Guaicol peroxidase
 GPX- Glutathione peroxidase
 GR-Glutathione reductase
 GSH- Glutathione
 GST- Glutathione-S- Transferase
 H₂O₂-Hydrogen peroxide
 H₂O-Water
 H₂SO₄ -Sulphuric acid
 H₃BO₃-Boric acid
 HCl-Hydrochloric acid
 HgCl₂-Mercuric chloride
 HPLC- High performance liquid chromatography
 kDa-Kilo dalton
 KNO₃-Potassium nitrate
 KNO₃-Potassium nitrate
 MDA- Malondialdehyde
 MDHR- Monodehydroascorbate Reductase
 MgCl₂-Magnesium chloride
 MgSO₄.7H₂O-Magnesium sulfate heptahydrate
 mL- Milliliter
 mM- Millimolar
 Mn- Manganese
 MnSO₄.H₂O – Manganese sulfate monohydrate

MPa- Megapascal
 $\text{Na}^+ \text{-K}^+$ tartarate-Sodium potassium tartarate
 Na_2CO_3 -Sodium carbonate
 Na_2EDTA - Di -Sodium ethylene diamine tetra acetic acid
 Na_2MoO_4 -Sodium molybdate
 NADPH- Nicotinamide adenine dinucleotide phosphate
 NaHCO_3 - Sodium bicarbonate
 NaNO_2 - Sodium nitrite
 NaOH-Sodium hydroxide
 NaPO_4 - Sodium phosphate
 NBT -Nitroblue tetrazolium chloride
 $\text{NH}_4\text{H}_2\text{PO}_4$ -Ammonium dihydrogen phosphate
 N-Normality
 O_2 - Oxygen
 $\text{O}_2^{\cdot -}$ -Superoxide radicals
 OD-Optical density
 PAGE- Polyacrylamide gel electrophoresis
 PCD- Programmed cell death
 PEG- Polyethylene glycol
 PMSF-Phenylmethanesulfonyl fluoride
 POX-Peroxidase
 ROS-Reactive oxygen species
 RWC-Relative water content
 SDS- Sodium dodecyl sulphate
 SOD-Superoxide dismutase
 TBA- Thiobarbituric acid
 TBARS- Thiobarbituric acid reactive substances
 TCA- Trichloroacetic acid
 TEMED -N,N,N',N'-Tetramethyl ethylenediamine
 Ti-Tolerance index
 UV-VIS-Ultraviolet -Visible
 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ – Zinc sulfate heptahydrate
 Zn-Zinc
 α -TOC - Alpha tocopherol
 μM - Micromolar
 $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ - Ammonium molybdate tetrahydrate
 $\bullet\text{OH}$ - Hydroxyl radicals

APPENDIX C: List of Chemicals

0.05M Sodium Phosphate Buffer (pH 6.8)
0.05M Sodium Phosphate Buffer (pH 7.2)
0.1M Potassium Phosphate Buffer (pH 7.6)
0.2 M Sodium Phosphate Buffer (pH 5.4)
1.0M Tris buffer (pH 6.8)
1.5M Tris buffer (pH 8.8)
2, 2-Diphenyl-1-Picrylhydrazyl
3, 3'-Diaminobenzidine
52% Perchloric Acid
80% Ethanol
95% Ethanol
Acetone
Acetonitrile HPLC grade
Acrylamide
Ammonium Dihydrogen Phosphate
Ammonium Persulphate Solution
Anthrone's Reagent
Ascorbic Acid
Benzidine
Boric Acid
Bromophenol Blue
Calcium Nitrate Tetrahydrate
Concentrated Sulphuric Acid
Coomasie Brilliant Blue
Copper Sulphate
Copper Sulphate Pentahydrate
Di sodium Ethylene Diamine Tetra Acetic Acid
Diethyl Ether
Ethylene Diamine Tetra Acetic Acid
Ferric Chloride
Ferrous Sulphate
Folin Ciocalteu's Phenol Reagent
Glacial Acetic Acid
Glucose
Glutathione
Glycine
Hexane
Hydrochloric Acid
Hydrogen Peroxide
Liquid Nitrogen
Magnesium Chloride
Magnesium Sulfate Heptahydrate
Manganese Sulfate Monohydrate
Mercuric Chloride
Methanol
Methionine

N,N,N',N'-Tetramethyl Ethylenediamine
N' N' Methelene Bis-acrylamide
Nelson's Arseno Molybdate Reagent
Nicotinamide Adenine Dinucleotide Phosphate Reduced Tetrazolium Salt
Ninhydrin Solution
Nitroblue Tetrazolium Chloride
Ortho-Dianisidine
Phenyl Methanesulfonyl Fluoride
Polyethylene Glycol
Potassium Di-Hydrogen Phosphate
Potassium Ferricyanide
Potassium Hydrogen Phosphate
Potassium Nitrate
Proline
Riboflavin
Sodium Carbonate
Sodium Dihydrogen Phosphate
Sodium Dodecyl Sulphate
Sodium HydrogenPhosphate
Sodium Hydroxide
Sodium Molybdate
Sodium Nitrite
Sodium Potassium Tartarate
Sodium Sulphate Anhydrous
 β -Mercaptoethanol
Sucrose
Sulfosalicylic Acid
Thiobarbituric Acid
Titanium Sulphate
Toluene
Trichloroacetic Acid
Tris
Tris-HCl(pH- 6.7)
Tris-HCl(pH-8.9)
Tween 80
Zinc Sulfate Heptahydrate
 α '- α ' Dipyridyl Solution