ENHANCEMENT OF SEED VIGOUR AND VIABILITY OF AROMATIC RICE BY USING CHEMICALS UNDER CLIMATIC CONDITIONS OF DARJEELING HILLS

A Thesis submitted to the University of North Bengal for the Award of Doctor of Philosophy in Botany

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DECLARATION

I declare that the thesis entitled **"ENHANCEMENT OF SEED VIGOUR AND VIABILITY OF AROMATIC RICE BY USING CHEMICALS UNDER CLIMATIC CONDITIONS OF DARJEELING HILLS"** has been prepared by me under the supervision of Dr. Projjwal Chandra Lama, Assistant Professor of P.G. Department of Botany, Darjeeling Government College. No part of this thesis has formed the basis of any previously awarded degree or fellowship.

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This is to certify that the thesis entitled **"ENHANCEMENT OF SEED VIGOUR AND VIABILITY OF AROMATIC RICE BY USING CHEMICALS UNDER CLIMATIC CONDITIONS OF DARJEELING HILLS"** submitted to the University of North Bengal, Rajarammohan Pur, Darjeeling, West Bengal for the award of degree of Doctor of Philosophy in Botany, embodies original research work carried out by Miss Deepa Tamang, M.Sc under my supervision. This work has not been submitted in part or in full for this or any other degree before.

Miss Tamang has followed the rules and regulations as laid down by the University of North Bengal for the fulfilment for requirement for the award of the degree of Doctor of Philosophy in Botany. The results incorporated in this thesis have not been submitted for any other degree.

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ABSTRACT

In the Darjeeling Hills, the cultivation of aromatic rice is indigenous and organic by default. Rice is known as "dhan" in the local language (Nepali). Based on indigenous farmers' perceptions, traditional landraces, commonly grown local cultivars, have superior traits in terms of survival, seed care, and harvest joy.

The experiment for 0-, 90-, 180-, 270-, and 360-day long-term accelerated ageing (95% RH, 2–18°C) was related to a proportionate loss in proteins, RNA, insoluble carbohydrate, and dehydrogenase enzymes in the seed kernels of aromatic rice as well as a decrease in the TTC-stained seed percentage. Control samples with the identical seed materials under long-term accelerated ageing (95% RH, 2–18ºC) showed dramatic alterations, and all of the seed samples were attacked by storage fungi of various species.

The ASA pretreatment, SADH pretreatment, and Na-dikegulac (2,3,4-6-di-O-isopropylidene-L-xylo-2, hexalophuranosate) pretreatment of seeds significantly controlled the decrease in levels of certain vital cellular components such as carbohydrates, protein, RNA, DNA, and dehydrogenase enzymes as well as the TTC-stained percentages. This research has shown the long-term accelerated ageing experiment to be critical to increasing ageing damage in control seeds. The results of this study have shown that chemical pretreatment is effective in accelerating ageing damage in the overall cellular metabolism, providing substantial alleviation. In comparison to the ASA, SADH, and the NaDK, they have been conclusively proved more effective in the accelerated ageing experiments of this investigation.

The percentage germination of rice seeds steadily decreased as accelerated ageing (95% RH, 2-18ºC) progressed from 0-, 90-, 180-, 270-, and 360-days, regardless of the concentration of chemical pretreated samples or control samples. The decline in seed germination was, however, significantly more apparent in the control set. All chemical concentrations effectively slowed down germination, and in the latter observational periods, the impact of NaDK was shown to be significantly more important. The parallel suppression of the field emergence of seed in both the control and chemical pre-treated seed samples was connected with the ageing-inducing reduction in seed germination. Seed pretreatment with chemicals, on the other hand, partially mitigated the ageing-induced impairment of field emergence.

As a result of the 360-days of accelerated ageing, the loss of seed germination and field emergence ability led to a significant slowdown in the speed of germination in both control and chemically pre-treated aged samples. This is demonstrated by the fact that, even after a 172-hour germination time, almost no seeds germinate. In controlled seed lots under accelerated ageing conditions, the pace of germination was found to have significantly slowed down with increasing age. On the other hand, the pretreated chemicals ASA, SADH, and NaDK, however, have been proven to withstand the harmful effects of accelerated ageing and retain germination speed in the seed lots. The accelerated ageing treatment had a significant inhibitory effect on seed germination behavior. Seed pretreatment with NaDK prior to accelerated ageing treatment was shown to partially counteract this inhibitory effect.

The metabolism status of the seeds was also altered, as evidenced by alterations in nucleic acids, soluble carbohydrates, amino acids, and the activity of dehydrogenase, amylase, IAA, catalase, and protease enzymes inside the seeds. Regardless of control or chemically pretreated seed samples, DNA levels in seed cotyledons were shown to decrease as ageing time increased. However, in chemical pretreated samples, the degree of the decrease was significantly less, and yet, with the progression of ageing time in chemical pretreated seed samples, decreasing RNA levels were also discovered. The quantities of carbohydrate, amino acids, dehydrogenase, and catalase in seed ageing decreased over time in control samples, but this tendency was somewhat stopped by the pretreating chemicals. In chemical pretreated seed samples, the activity of soluble carbohydrate, IAA, and protease enzymes increased with the seed ageing process throughout the observation periods. However, in seeds that were pretreated with Na-dikegulac, the rate of increasing activity was observed to be modest. Amylase activity, on the other hand, steadily decreased with seed age.The speed of decreasing enzyme activity was slowed down by Na-Dikegulac.

Plant growth and development were hampered when plants were produced from seeds that had undergone an accelerated ageing treatment (95% RH, 2-18^{o}C) for 360 days. Plant height, stem circumference, dry matter content of intact plants, and plant growth phases are all inhibited by ageing. As seen by substantially higher values of the growth performance recorded at seedling stage, total seed development on panicle, fully filled grain, and seed pretreatment with ASA, SADH, and Na-dikegulac, at least partially alleviated such an ageing-induced inhibitory effect on growth performance. Regardless of the decreasing

activity of enzymes, it has been determined that Na-dikegulac is more effective and provides better results than control, ASA, and SADH.

Some vital cellular components in leaves, such as RNA and soluble carbohydrates, as well as the activities of catalase, superoxide dismutase, IAA-oxidase, RNAse, and protease enzymes, were clearly reflected in plant metabolism as an accelerated ageing-induced hindrance on plant growth performance in the field.

All five developmental stages, pre-flowering stage (P), flowering stage (F), seed forming stage (S), seed maturation stage (M), and pre-harvesting stage (H), of plants produced from seeds that were subjected to an accelerated ageing treatment for 360 days showed significant reductions in DNA, and soluble carbohydrate levels in leaves decreased first in three subsequent sampling periods (P, F, and S). However, there was a sharp rise in the next two following stages (M and H). The result was in contrast to RNA and insoluble carbohydrate levels. The chemical relief of such an inhibitory impact on plant metabolism was obvious by arresting the decline in DNA and maintaining the levels of soluble and insoluble carbohydrates and RNA concentrations in leaves. In addition, the chemical effectively decreased the age-related loss of catalase and superoxide dismutase enzyme activities in leaves, as well as the greater activities of the catabolic enzymes IAA-oxidase and protease.

Accelerated ageing of seeds has led to a delay in the growth and development of plants, which has led to an extension of the life cycle. This slowed overall growth and was visible at every step of plant development studied, including radical emergence, leaf emergence, internodal elongation, and so on. Crop production was affected by changes in the general metabolic status of seeds, seedling development, and plant metabolism, as evidenced by assessments of some yield variables such as total filled seeds per panicle and 1000 seed weight. Seeds that were subjected to an accelerated ageing treatment performed poorly in the field and had low growth potential, particularly in control plants. As a result of the slow overall plant development, crop output was harmed, as seen by the decrease in total seed yield per plant, total filled seeds per panicle, and 1000 seed weight. In comparison to control seed lots, which had received previously accelerated ageing treatment, the pre-treating chemicals, regardless of their concentration, demonstrated better germination percentages. Na-dikegulac efficiently improved harmful effects on the behaviour of germination of seed,

seedling development, and metabolism, as well as accelerated harmful effects generated by ageing on seed.

The positive effect on germination behaviour *was* induced by chemical pretreatment under stressful conditions, i.e., accelerated ageing was clearly evident in seedling development and metabolism. This was apparent because the chemical NaDK significantly induced larger seedlings and dry matter content of undamaged seedlings, the stopping of amylase and protein loss, and the activity of catalase and superoxide dismutase enzymes in contrast to control and other chemicals.

The difficulty of the vigour tests and ageing changes has been to establish quantifiable parameters common to deteriorating seeds depending on their ageing, variations between crops or circumstances of harvesting. There has also been a thorough examination of factors linked to rice seed quality in terms of accelerated ageing and varietal variations in the rice seed longevity stored in ambient settings. As a result, it was deemed necessary to explore physiological and biochemical alterations in order to comprehend the causes of seed deterioration. This helps discover not just causes to improve seed storage, but also details to help incorporate features to improve the storage quality of high-performance varieties. Thus, the present investigation was aimed at investigating various physiological and biochemical changes during seed deterioration as a result of accelerated ageing as well as finding the superior variety among five experimental varieties of rice (*Oryza sativa* L.) and the favourable benefits of Na-dikegulac on maintaining increased seed storage potential, seedling performance, plant field performance, and plant tolerance to harsh environments are described.

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Introduction

1. INTRODUCTION

Seed Deterioration (Physiology and Biochemistry)

Storing of seeds is a major issue in tropical and subtropical nations like India, where seed ageing, degradation, and non-viability are significantly accelerated, mainly by high temperatures and high humidity (Dey *et al*., 2012). In rice cultivation, high relative moisture and high temperatures, which cause rapid seed degradation, are depicted. A natural phase of seed deterioration is the uniformity loss, viability, and vitality as a result of age or exposure to severe environmental circumstances. The degenerative process is irreversible and occurs during storage. The degradation of seeds in agriculture and horticultural production is an important concern. This is a typical catabolism that terminates the seed life, leading to total viability loss. The pathogenic attack also enhanced the process as an adverse environmental condition. Special consideration is given to the seed technologies, crop growers, and seed men related to the seed sector. This inevitable natural detrimental process, in particular to pathogens and environmentally harmful accelerated ageing, leads to rapid seed deterioration.

Economically, seed deterioration is a major problem for agricultural production. Therefore, agriculturists and horticulturists are regularly crippled when it comes to maintaining standard seed vigour under environmental storage conditions. It is also reported that certain manipulative approaches such as X-ray radiation, hydration, dehydration, etc. can increase the lifespan of seeds or pre-treat seeds using a variety of chemicals such as phenols, salts, organic acids, hormones, and vitamins before storage (Coolbear, *et al*., 1984; Bhattacharjee, *et al*., 1993; Pati, 2020). Previous perceptions of some sodium-dikegulac (Na-DK) growth retardants, in particular, had a significant retroactive effect on seed senescence in storage. As a result, an antioxidant, ascorbic acid, a phytohormone, IAA, and a volatile oil, basil oil, were used as chemical manipulative agents to delay seed senescence, along with a growth retardant (Na-DK) (Bhattacharjee, *et al*., 1984 & 1993; Rai, 2000; Ojha, 2014; Lama, *et al*., 2016; Bhattacharjee, *et al*., 2018; Kanp, *et al*., 2021). The physiological and biochemical characterization of seed degradation continues to be the most ignored field of seed research. This lack of experience is primarily due to the many occasions leading to loss of seed viability, which leads to adversity in determining the cause and effect of a particular seed deterioration response (Copeland and McDonald, 1995).

Artificial Seed Deterioration Technology

Seed attributes are one of the pivotal factors that influence the quality and quantity of yield. Seed vigour is a vital component of seed quality. Seed deteriorative response typically initiates at physiological maturity and persists throughout harvest, processing, and storage, which are greatly influenced by genetic, production, and environmental aspects. The process of deterioration is predominantly a gradual and consecutive process where oxidation of fatty acid chains within the phospholipids of the membrane is the earliest change that is observed during cell membrane deterioration (McDonald, 1999). Deterioration of cell membranes prompts a reduction in the amount of ATP produced as an energy source as well as decelerates the synthesis of specific enzymes fundamental for growth and development, decreasing respiration, which provides energy molecules required for seed growth. The accumulation of all these deleterious changes contributes to a gradual decrease in seed vigour a decline in germination rate, a loss in storage capacity and the ability to resist diseases. In the long term, this perceptual manifestation of seed quality loss is manifested by an increasing incidence of anomalous seedlings, reduced speed and consistency of field emergence, and ultimately reduced crop yield (AOSA, 1983; Chhetri, 2009).

Numerous measures have already been developed to quantify vigour decline on the basis of physiological ageing impact, and the commonly acknowledged criterion of degradation of seeds is reduced germination (Malik & Shamet, 2009; Khan, *et al*., 2007; Jatoi, *et al*., 2004; Woltz & Tekrony, 2001; ISTA, 1993). Accelerated ageing, an easy and effective approach to assessing the degradation sequencing and relationships over a short time span by exposing seeds to high temperatures and high humidity levels, is a key approach. For some crop varieties, accelerated ageing research protocols have been standardized, as in soybean seeds (Krishnan, *et al*., 200; Sung, 1996; Tekrony, 1993), pea seeds (Jatoi, *et al*., 2004), chickpea seeds (Maeda & Wutke, 1996; Kapoor, *et al*., 2010), corn seeds (Tekrony & Woltz, 2001), pigeon pea seeds (Rao & Kalpana, 1995), radish seeds (Jain, *et al*., 2006), rice seeds (Millati, 2019; Bhattacharya, *et al*., 2013; Bam, *et al*., 2006; Zhou *et al*., 2002; Ellis *et al*., 1992; Ray *et al*., 1990; Sheshu & Krishnaswamy, 1990).

The Tamil Nadu Rice Research Institute conducted an investigation.The results of the experiment showed that chemically treated seeds with the anti-oxidant tocopherol (Vitamin-E) at 1% for 18 hours and kept in a gunny bag showed higher germination (95%) and seedling vigour compared to control, but after six months, germination and vigour

significantly decreased regardless of the treatments (Sasikala *et al*., 2018). The *Seleta* seeds needed for commercialization maintained their germination above the minimum regardless of the environment (Yogalakshmi *et al*., 1996; Marques *et al*., 2014). The effect of ageing is attributed to a decrease in viability percentage, which leads to the depletion of food reserves and a decrease in the unnatural activity of embryos. The ageing of seeds results in the production of free radicals, which can be controlled by antioxidant treatments such as ascorbic acid, tocopherol, and glutathione (Draganic and Lekic, 2012).

Aromatic Rice of Darjeeling Hill – Details and Importance

Characteristic Features of Aromatic Rice

Every state in the country has its own rice variety and quality/specialty. Farmers mainly cultivate fine, short, aromatic, highly scented grain varieties of rice for their own consumption and ceremonial purposes in their areas in West Bengal, Orissa, Chhattisgarh, Bihar, and the North East states. These varieties have a weak stem, a long growth period, a low grain weight, and a low yield, and their market is underdeveloped. There is a different class of aromatic rice, which is long-grained with a special blend of grain, cooking and eating consistency. In the north-west part of the Indian subcontinent, these rice varieties are best represented in the quality traits. Basmati rice is traditionally produced and exported by India and Pakistan, and it sells for three times as much as high-quality non-Basmati rice on the international market.

The Sanskrit words vas (aroma) and mayup (ingrained or present from the start) were combined to form the word "Basmati," which means "ingrained aroma." There is a common misconception that any aromatic rice is Basmati, but this is not the case since the fragrance/aroma is unique to Basmati, as is the silky texture of the long Basmati grain, which is unmatched by any other rice grain. There is no particular standard that can differentiate Basmati rice from other aromatic rice grown anywhere else in the world. Since time immemorial, several scented rice varieties have been grown in the Indian subcontinent. View texture and aroma intensity with minimal kernel dimensions, linear kernel elongation with minimal swelling, fluffiness, appetizing, fast digestibility, and longer shelf-life of cooked rice are the consistency combinations that make Basmati rice more appealing to consumers and traders (Shamimagrimet, 2013).

Rice has been exceptionally valued in Indian culture for many years because of its outstanding consistency and is considered auspicious (Bhattacharjee *et al*., 2001). These highly regarded rice varieties are known across Asia, as well as Europe and the United States, as "Basmati" (bas=aroma). Aromatic rice was traditionally cultivated in India's Himalayan foothills, and its name is therefore historically connected to its topographical origin (Bligh, 2000). Superfine, long, slender grains with a calcareous endosperm and a pattern similar to something like a Turkish blade define high-quality aromatic rice. cooked rice with a pleasant and unusual aroma, sweet flavor, dry, soft, and delicate texture, fine curvature, less amylose, medium to low gelatinization temperature, delicacy, and 1.5 to 2 times length-wise elongation with the least breadth-wise swelling (Siddiq *et al*., 1997).As a consequence, the global economy exorbitantly prices all of these Basmati rice properties. However, after cooking, flavor, and rice grain elongation, the scent is considered the most desired attribute by Indian farmers and customers.

Climatic Requirements

The quality of Basmati rice production is dependent on consistent irrigation, drainage, and regular soil. High humidity (70-80%) and a temperature range of 25–35 °C are ideal during the vegetative growth season. Bright and clear days with temperature ranges of 25–32°C, relatively moderate humidity, low wind intensity, and cooler nights (20–25°C) are considered important for proper grain and aroma production during flowering and maturity. The 1st worldwide final fine-grain "Aromatic Rice Observational Nursery Trial," IRRI-coordinated and including 26 sites, recently elevated the standard of aromatic rice to an international level. This preliminary included thirty Indian and Pakistani cultivars, including Basmati (Singh *et al*., 1997). Regardless of the variety or size of the kernels, scent or natural aroma is assigned a premium price and is valued almost uniformly in both foreign and domestic market sectors. Basmati rice is the most commonly desired and commands the highest premium pricing in the finest preparations such as Biryani and Pulao almost anywhere in the world.

Geographic Description

Darjeeling district is located in West Bengal, with a portion of the district falling within the northern zone (approximately 2.43 lakh ha) and a portion falling within the terai-

Teesta alluvial zone (approximately 12.15 lakh ha) (Anon., 2009a; Adhikari *et al*., 2011). Rice grain is an important staple food grown in West Bengal and has long been associated with a variety of social festivities and ceremonies in Bengal life since time immemorial. West Bengal has the most diverse rice biodiversity and is hence known as the country's rice bowl. The rice ecospecies are so diverse and distinct that they have developed impulsively into a state whereby scientists instantly name them *Oryza sativa* var. benghalensis (Chatterjee *et al*., 2008). Since then, rice has been farmed all throughout the state since then, and the grain has now become an integral part of Bengal‟s life. It is not just the basic cuisine; it has also been mixed with rice in various cultural ceremonies and celebrations.

Darjeeling town is located at an average elevation of 6,710 feet in the Lesser Himalaya (2,045.2 m). Darjeeling is a triangular shaped district in Western Bengal ranging from north latitude to 26°31', north latitude to 87°59', and eastern longitude to 88°53'. Rice cultivation in the Darjeeling Hills, which is naturally organic, has a yield of just 9 q/ha. The poor yield of rice in this area is due to the erosion of the soil. Little is added to it and it continues to grow plants annually. There is no effort to recover and boost the soil's nutritional state (Rai, 2008). In the region, the subtropical humidity with rainfall varies between 2500 and 3500 mm. The maximum is 19.5^0 C and the minimum is 4.8^0 C (yearly average). Rainfall in this location is distributed between March and May at a rate of 398.5 mm, from June through October at 2637.5 mm, and 68.5 mm between November and February (Adhikari, *et al*., 2011).

In this area, the aromatic rice varieties are extremely short, thin-grained, and profoundly fragrant. Each variety is expensive in the area where it is produced, and it lacks a well-established market. These varieties are distinguished by their weak stems, very long growth time, low grain weight, and poor yield. Farmers consider rice as auspicious and basically cultivate rice for personal domestic consumption and utilisation for festivity and auspicious ceremonial purposes like pujas, weddings, and so forth.

The people of Darjeeling Hill have a wealth of knowledge useful to the neighbourhood in cultivating the local environment because of their familiarity with natural farming. Darjeeling Hill is home to a wide range of languages, as well as a long history of social and cultural identity characterised by differences in customs, cultures, and practises. The villagers engage in traditional hill cultivation, where fields are rain-fed and cultivating

frames are constructed using various indigenous techniques. Maize, corn, and vegetables such as cabbages, potatoes, squash, coriander, and chillies are among the crops planted. In the hills, cultivation systems are inseparably linked to usability in terms of the comparison of usability and use of assets (Mukherjee, 2012).

Rice has been grown in Darjeeling for many years in a very small field. Indigenous methods of agriculture and innate scientific competence are part of a particular community's culture and history. Farmers rely on biodegradable waste in indigenous agricultural systems, which are effective in helping to maintain soil quality. Wet rice terrace agriculture, finger millets/black lentils, and forest area management by eco-friendly ecosystems and bioregional farm activities such as bio-waste recycling are all part of the rice-based farming scheme, which requires upgrading the advancement process to benefit from these populations (Mukherjee, 2010).

Panikheti

"Panikheti" is an indigenous rice cultivation technique that is practised by people at lower altitudes in the Darjeeling Hills. The terrace is filled with water in this farming scheme, which is redirected from the hilltops to make tiny shrubs like lemongrass and citronella flourish on the terrace that regulates the erosion of water. As soon as stream water rises from the hill forest, it is caught and canalised at the valley's rim, and diverted into primary, secondary, and tertiary network channels. Soil nutrients in hilly regions are controlled by weeds and other biomass that is generated on the soil, and people usually use cow dung and urine for treatment of soil-born insects and plant pathogens. These mechanical steps are an environmentally sustainable system for preventing water erosion in the landscape.

Present Study

Aromatic Rice

Rice, "one of the major developments in the history of mankind," is a cereal food that constitutes an essential part of a diet that produces cereals or grains, the second most consumed in the world. It is consumed as a basic food by over 60% of the population and is successfully grown in many parts of the world, including dry land mountain slopes, wetland valley bottoms, and terraced fields in hills. In the international market, Indian flavours and

the appropriateness of rice are highly valued. Rice provides good protein and carbohydrate sources, as well as fibre, vitamins, and minerals. It is also a good source of energy and gives humans 15% of the world's protein and 21% of the world's calories per capita (FAO, 2003). India, the world's second largest rice producer (116.58 mt.), has a total rice area of 42.17 million hectares, or 33.3 percent of the total rice production area (Venkataramani, 2002).

Screening

Due to its extremely high relative humidity, which promotes the growth of microorganisms, the question of maintaining seed vigour in Darjeeling and the territories surrounding it is far more serious. As most crop seeds probably need storage for either one or a few planting seasons, farmers and horticulture professionals from this area are often impeded from maintaining standard seed vigour in an environmentally friendly storage environment. The moisture content of seeds and the storage temperature deeply affected the deterioration rate (Rai, *et al*., 2000 &1993; Ellis, *et al*., 1992). Seed degradation has been shown to have several physiological and biochemical indicators (Saraswathy *et al*., 2017; Kapoor *et al*., 2010; Jatoi *et al*., 2004; McDonald, 1999; Kruse, 1999; Kalpana and Rao, 1995).

Scientific Study

The storability of rice seeds is a key feature of the programme for the storing of healthy seeds when the crop is well preserved in the processing process. According to preliminary findings (Akter *et al*., 2015), rice yields may be increased by 10–15% simply by using good and stable seed. If processed under adverse climatic conditions, high quality seeds can even severely deteriorate. Long-term storage of rice seeds entails a significant amount of effort to protect the seeds from distinct climatic disasters such as extreme summer, winter, monsoon, and so on, all of which are incompatible with producing stable and well-developed rice plants. There have been many studies on rice seed storage in different parts of the world under various storage conditions (Khalid *et al*., 2001; Kapoor *et al*., 2011; Baek *et al*., 2018; Henge, *et al*., 2019). During storage, however, the vigour and viability of the seeds depend mainly on the genotypes and conditions of processing and post-harvesting, such as the seed quality, conservation packaging, air and storage temperature, relative moisture content, exchange of gas and skin characteristics of the seed.

Causes of Viability Lost

When processed in a natural environment, rice seeds display low physiological consistency. Tropical weather conditions with high relative humidity and temperature, along with unfavourable storage conditions, inevitably contribute to complete seed quality loss by affecting the storability of cereals, oilseeds, etc. (Marques, *et al*., 2014; Begum, *et al*., 2013;). Rice seeds stored for a half year in earthen, tin, and plastic holders without controlling temperature and relative humidity showed zero germination and minimum germination in the case of seeds stored in a gunny bag (Sultana *et al*., 2016). There is no doubt that proper seed treatment and storage techniques will greatly enhance seed quality and significantly increase the yield. Similarly, seed treatment with antioxidants such as ascorbic acid, tocopherol, and glutathione, as well as the use of bio-fungicide, has been found to have a beneficial effect on seedling vigour and is superior in increasing germination and vigour in rice seeds (Hossain and Akter, 2015; Lekic and Draganic, 2012; Basra, *et al*., 2006).

Adverse Climatic Condition

In 1965, in order to determine the storage capacity of seeds and to determine the vigour, vitality, and durability of storage seeds, Delouche first developed an accelerated ageing test (Khan, *et al*., 2017). The technology includes resistance to unfavourable temperatures of the seeds and 100% R.H. After a period of time, a normal germination test is performed. Owing to the high temperature, the seeds quickly aged and absorbed moisture from the humid environment. The precondition for the test is that high temperatures and high humidity in the most robust seeds maintain their capacity for natural germination in test seedlings. Later experiments confirmed the correctness of this test in order to determine the shelf life of different seed species under different conditions of storage (Delouche and Baskin, 1973). Accelerated ageing tests were proposed for stand establishment and indicated that this test may have added benefits, other than storage capacity, to predict seed yields (Baskin, 1970). Several analyses of the impact of the accelerated ageing test on crop seed field emergence have shown that the accelerated ageing test will predict field emergence. Further research has shown that the accelerated ageing test is also effective in forecasting the establishment of different crops' seeds.

Manipulator to Overcome the Adverse Condition- Phytohormones

Seed treatment and priming are two important quality aspects of seeds that can improve stand seed quality, increase yields, and protect against pests (Powell, 2006; Khan, *et al*., 2017). Rice seeds pretreated with sodium dikegulac (NaDK) at 1,000 and 2,000 µg/ml slowed germination, stopped electrolyte leakage, decreased soluble carbohydrate leaching, and slowed the rate of RNA loss from seeds (Tamang *et al*., 2020; Pati *et al*., 2019 & 2018; Bhattacharjee *et al*., 1994 and Bhattacharyya, 1989).

Probable Outcome of the Present Study

Since 2000, it has been estimated that 10 billion people depend on rice as their primary food source. As a result, rice consumption and usage have risen faster than global rice supply, and demand is expected to hit about 880 Mt. More than 3/4 of the population lives primarily in rural areas, and approximately 1 billion families rely primarily on rice farming and agricultural jobs for a living. If the population's consumption of rice continues to rise, we will face a future problem as the amount of land and water required to grow rice decreases, and the excessive use of fertilizers, pesticides, and insecticides has a negative impact on the environment. Rice research science and technology advancements are becoming increasingly important in order to improve rice production and sustainably agricultural growth.and cooperation in the scientific community as well as determination and accountability among all parties involved would be needed to ensure a steep increase in sustainable rice production. By 2030, Asia's paddy rice yield is expected to increase by 11.7 to 23.4 percent, reaching 4.59 to 5.08 mt/ha 1. As a result of technical advancements and more productive use of knowledge sources combined with increased cultivation area, productivity may increase over time. Rice production could change as a result of global warming due to changes in CO_2, temperature, and precipitation. Consequently, policymakers require accurate provincial production effect forecasts to take account of procedures of alleviation and adaptation. A wide range of Asian nations and organisations have agreed to strengthen national strategic initiatives and budgetary assistance for science, seed development, food safety, and hybrid rice growth (FAO, 2001). FAO has improved the method for determining the effects of changes in productivity on postharvest strategies for many crops, including rice (Mejia, 2015).

Chapter II

Review of Literature

2. REVIEW OF LITERATURE

This survey was conducted as part of a comprehensive project on aromatic rice, with the aim of improving the vigour, viability, and post-harvest loss of selected plants grown in the Darjeeling Hills, as well as preventing seed deterioration and improving germinating efficiency and productivity. This analysis contains a brief introduction to aromatic rice, with a focus on the global scenario and the topics covered in this study.

Seed Science and Technology

The study was conducted on the degradation, storage, and viability of chemical manipulation of the rice grains, as well as post-harvest analyses of aromatic rice. The emphasis was on chemically induced improvements in the consistency and productivity of aromatic rice seeds, as well as the investigation of specific phytochemical pretreatment. According to the literature, certain chemical manipulative agents are used to improve the efficiency and productivity of seed plant production in productive ways (Bhattacharjee, 1984; Rai, 2000; Kanp, 2007; Dolui, 2008; Sultana, *et al*., 2016; Saraswathy, *et al*., 2017, Pati, 2018; Zhou, *et al*., 2020).On various aspects of aromatic rice research, the current review focuses, including postharvest system, seed viability, growth modulation, metabolism, efficiency, and phytochemical analysis.

Three methods of hydration-dehydration treatment in mid-storage, such as soaking, drying, moisture equilibrium-drying, and moist sand cooling-drying, each with different durations, have been used to assess the vigour and viability of stored pea seed (*Pisum sativum* L.). Maintaining membrane integrity and preventing lipid peroxidation, despite the proven harm of soaking-drying, and all durations of moisture equilibration treatment except 120 h outperformed the regulation, while 48 h proved to be the best, could be plausible explanations for such beneficial results (Ramamoorthy *et al*., 2008). Pretreating pea seeds with chemical substances decreases the germinability loss greatly. In chemical pretreatment, pea seed performance was also shown to be significantly improved, and thus the yield of healthier plants increased (Pati *et al*., 2017).

Naturally, seeds of *Allium cepa* L. are less feasible and hydrophilic than many other seed plants. Today, Tamil Nadu is boosting the production of seed onions in India. Inadequate stockpiling of onion seed or disadvantageous circumstances results in degradation

of seeds marked by a lack of viability, vigour loss and seed uniformity loss. Onion seeds can be kept germinating and growing for more than a year, lowering to 6 ± 1 percent the seed moisture content and conserving it between 4 to 15°C and relative humidity 40 to 60 percent in damp sealed receptacles. Seed invigoration treatments such as hydration-dehydration, different pretreatments were discovered to be effective in delaying seed deterioration and, as a result, the viability and survivability of seeds during storage is increased (Saraswathy, *et al*., 2017; Jangjoo, *et al*., 2020).

The impact of soaking on seed vigour and viability in artificial ageing conditions and the effects of hydrate dehydration on viability and storage were examined for tomatoes and radish seeds. During mid-storage, hydration-dehydration of seeds was successful in increasing seed retention. Presoaking of seeds with water followed by drying was subjected to rapid ageing (40Q C, 90 percent RH for 48 hours) and storability demonstrated greater seed germinability and vigour than unsoaked seeds in both the tomato and the radish mainly affected by moisture content and environmental factors such as temperature and relative humidity during storage. Certain toxic compounds accumulate in seeds, shortening the storage time, though it has been discovered that storing seeds with water or chemicals improves seed germination and activity (Pan and Basu, 1985; Doijode and Raturi, 1990; Kim *et al*., 2006; Kar *et al*., 2011; Dominic, *et al.,* 2016).

Aromatic/Basmati Rice

Basmati rice, also known as the scented pearl, is a blessing from nature that is exclusive to the Indian subcontinent (Bhattacharjee *et al*., 2001) and is well-known for its abundance of basmati and aromatic non-basmati rice grown on its own land. Aromatic short grain varieties are inexplicably exclusive for scent, excellent cooking, and consistency characteristics, which have a significant impact on worldwide customer choice and a high price command both domestically and internationally (Rani, *et al*., 1998, Khan, *et al*., 2003; Singh, *et al*., 2012). The export restriction for basmati and short-grain aromatic rice was put in place by India in 2011-2012, exporting 4000 thousand tonnes of basmati rice and earning Rs 10582 crores ($2.17 billion) in foreign exchange (FAO, 2012; Anonymous, 2012; DRR, 2014).

Rice, which has been called "one of the most significant developments in human history," is a cereal product that is an important part of the diet and the world's second-most eaten cereal/grain. It is consumed as a staple meal by about 60% of the global population. It is grown successfully on mountain slopes (or upland) and in wetlands in valley grounds and terraced terrain in different world regions. Rice of Indian flavour and suitability is highly regarded in the international market. Rice is a good source of starch and proteins, and it also has the best energy, vitamins, minerals, and fibres (FAO, 2003). There are 42.17 million hectares of total rice area (116.58 mt.) in India, accounting for 33.3 percent of the world's food crop area (Venkataramani, 2002).

International Scenario

The world's longest continuously cultivated cereal, rice, is "one of the most significant products in history," according to the International Institute for Rice Research (IRRI). The United Nations' Food and Agriculture Organization (FAO) proclaimed 1966 to be the rice year. The UN General Assembly designated 2004 as the "International Year of Rice" in significance recognition of this crop (IYR). IYR's theme "Life is rice" represents the value of rice in 2002 as the principal source of food.

The UN honoured rice as the only food crop twice, and the UN paid such a special homage to rice for the second time. Their enormous impact on social, economic, and political stability makes them considered a political good. Not only is rice a vital crop, it is also a key source of jobs and income for the rural poor and a key enriching element in society, lifestyles and ecosystem functions, a sign of cultural pride, global solidarity and existence (Shamimagrimet, 2013).

Phytogeographical and archaeological data indicate that rice from its wild ancestor, *O. rufipogon,* was domesticated more than 10,000 years ago. The Himalayan Mountain Ranges, which currently extend from East to South China, Nepal, Thailand, and Myanmar, contain *O. rufipogon* (Chang, 1976; Kush, 1997; Londo, *et al*., 2006).Rice has grown tremendously, but is essentially limited to Asia's monsoon. That is not the case for the worldwide distribution of wheat and maize. Rice is the most suitable lowland crop, where the drift of water naturally flows like runoff and rivers. As a result, a rare mixture of climate and landforms has aided in the development of Asia's paddy rice system.

Asian Scenario

Rice has nurtured more people than any other grain and has influenced billions of people in Asia's heritage, society, lifestyle, and economy. Whether it is to encourage social or community growth and promote war or pursue peace, generate prosperity or endure suffering, enjoy good health or live deficiently, or provide a basis for worshipping God, rice has been central to all facets of human life (Ahuja *et al*., 2001, O"Toole, 2004). This crop links India, China, Bangladesh, the Philippines, Burma, Thailand, Vietnam and all common rice nation countries and has since time immemorial been interwoven with Asian culture (Mitu De, 2014 & 2019).

Rice and its derivatives provide 60 to 70% of the calories consumed by over 2,000 million people in Asia alone.Its growth is crucial for food security and poverty reduction. Rice is grown on 159.40 million hectares around the world, with Asia accounting for 88.95 percent of global rice production and consumption (90.4 percent) (FAO, 2012; Singh *et al*., 2014).Rice cultivation employs approximately 300 million people in Asia and the Pacific alone, and it consists of two subspecies: *Oryza sativa* L. subsp. India, which originated in India, and *Oryza sativa* L. subsp. Japonica, which originated in Eastern Asia.

National Scenario

Rice is India's backbone, the second largest country to grow rice in an area of over 44.6 million hectares, yielding 104.32 million tones, averaging nearly 2.34 tonnes/hectare productivity, of which 85-90% is internally consumed by the Indian economy. Our national food safety is central to the development and prosperity of its supply for over 65 percent of the population (Anonymous, 2013, DRR, 2014, Rajasekar and Jeyakumar, 2014). India is the most produced region in Asian countries and has the largest proportion, accounting for almost 20 percent of the rice produced worldwide (Babu, *et al*., 2014). India is also one of the world's rice diversity hotspots, with significant inter-and intra-specific variation recorded (Roy *et al*., 2016). India has a plethora of specialty rice varieties, including basmati and short indigenous varieties that have become ingrained in Indian culture and are considered treasures (Talukdar *et al*., 2017). In addition, hundreds of indigenous short-grain aromatic and non-aromatic cultivars and landraces are cultivated in pockets throughout the states. Each state has its own aromatic rice stock in indigenous regions, which works well (Shobha Rani

and Krishnaiah, 2001). In addition to conventional varieties, India has many landraces and several lesser-known varieties that have been grown for years by both farmers and local entrepreneurs. The selection of such crops was based on desired characteristics, which led to the development of large agro-ecologically suited rice varieties and, therefore, nearly all Indian rice farming provinces have their own regionally accepted cultivars suited to distinct agro-climate factors and local outcomes (Singh, *et al*., 2003).

Aromatic (scented) Rice varieties in India and their distribution

TABLE: 1

States	Varieties (district)
Assam (65)	Rangafoha I, Joha 947 types), Bongali, Bhabeli, Kanjoha, Kanku, Khorikakala, Kopausali, Manki, Ranga, Rampal, Bagribhog, Tulsibhog, Govidbhog, Badshabhog, Prasad bhog, Malbhog, Kalajira.
Bihar (42)	Basmati3 (Patna); Katarani (Bhagalpur, Champaran) Kari bank (Patna, Bhojpur, Munger, northern Bihar) Mohindhan, Sagarbhog, Hansraj (Patna, northern Bihar); Sonachur (Bhojpur, Rohtas, northern Bihar); Badshahbhog (Bhojpur,Bhagalpur); Kanakjira (Bhojpur,northern Bihar); Shamjira (Rohtas, Aurangabaf, northern Bihar); Shapasand (Rohtas, northern Bihar); Tulsiphul (Rohtas,northern Bihar); Kanehonehur (Gaya); Mahijawain (Aurangabad, northern Bihar Tulsimanjari (Bhagalpur, Munger, northern Bihar); BR 9, BR10 (Bhagalpur, northern Bihar); Badshahpasan, Bahraini, BhuriC.basmati, Chenaur, Devtabhog, Kamod, Kalichamparan basmati, Kesarbani, LalC.basmati, Malbhog, Ramjawain, Sonalari, Tulsipas and (northern Bihar); Mircha, Malida, Satar (Muzzafarnagar); Amad, Abdul, Ramjain (western Champaran); Bramabhusi (Semara, Ramgarh, western (***Champaran); Deobhog(Darbangha); Kamini(Bhagalpur)***
Gujarat (5)	Pankhali, Kamod (Kheda); Krishnakamod (Ahmadabad); Kolhapur scented (Saurashtra); Zeersal
Haryana (2)	Basmati 370 (Rohtak, kaithal); Karnal local (Karnal,Kurukshetra, Panipat)
Himachal Pradesh (9)	Muskan, Ramhjawain, Achhoo ,Seond basmati, Baldhar basmati, Madhumalati, Chitru basmati (Kangra valley); Pansara local (Kullu); Hathkoti basmati (Shimla)
Jammu and Kashmir (7)	High hills: Gulzag,, Zagir, Muskkanti, Tumlazag; Mid-hills: Musk budji, Qadirbaig, Ranbir basmati (R.S. Pura, Katua, Jammu)
Karnataka (19)	Ambemohor (Belagoan, Dharwar); Devamallig (north Kanara), Gumsali (Haveri); Gandhsali, Gulvadi, Gamanasanna (south Kanara); Huggibatta

States	Varieties (district)
	(Belagoan, Dharwar); Jeerigesanna (Mysore, Bangalore, Kodagu, Chikmanglur); Kagisali (Balagoan, Dharwar, Haveri); Kumudh (Haveri); Karigajavile (Belagoan, Dharwar, Haveri); Krishnapasangi (Raichur, Gulbarga, Bellary0; KunsumKesari (north Kanara); Kalabatta (Tumkur, Banglore); Kavali (Bidar); Rattansagar (Bidar);Sindhagi local (Bijapur); VasaneSannaBatta (north Kanara); Yalakkisali (Haveri)
Kerala (7)	Gandhakasala, Jeerakasala, Velumbala, Chomala, Kayama (Wyanand); Kothampalari (Kannur); Pookkilathari (Palakkad); Amarjyoti (Mandalla); Adamchini, Antraved (Damoh, Panna); Badshahbhog (Bastara); Batanphul (Sidhi); Chakarbhatta (Chattarpur); Chhatri (Jabalpuur); Chindikapur (Raigarh); Chinoor (Balaghat); Chirnakhai (Bastar) Dilbaxa (Tikamgarh, Satna, Reva); Dubraj (Raipur, Durga, Rajnandgaon, Bilaspur,Mahasamund, Dhamtari, Janjgir, Korba, Kanker); Gangaprasad (Rajnandgaon); Kapursar (Rajpur, Durg, Rajnandgaon); Kubrimohr (Raipur, Durg); Loktimanchi (Bastar); Mekhrabhundha (Durg); Samodchini (Bilaspur, Surgujar); Kalimoonch, Ganju (Gwalior); Shakarchini (Surguja, Shahdol); Sri kamal (Shahdol); Tulsiamrit (Raigarh, Seoni); Laloo (east Madhya Pradesh); Vishnuparag, Tedai, Chinigauri, Chiranki, Kali kamod, Kaktimanchi, Mekrabidu, Vishnubhog, Banaspatri (pockets)
Maharashtra (6)	Ambemohor, Krishna sal (Pune, Satara, Ahmednagar); Banaspatri, Chinoor (Vidharbha); Gham (Raigad); Ghansal (Kolhapur)
Manipur (5)	Chakaoangouba, Chakaoamubi, Phorenmubi, Langgphouanganba, Chakaopoireiton
Mizoram (6)	Tai, Pharte, Bawangbuh, Mawangbuh, Zongam, Phanrai
Orissa (33)	Thakurbhog, Ratnasidol, Prabhatjeera, Nalidhan, Manasi, Jhinghasali, Sitakesari, Barangamali, Basnaphali, Jala, Jhilipanjiri, Lekhtimahi (Orissa); Kalajira (Cuttack, Puri, Ganjam, Koraput); Dubraj (Keonjhar, Deogarh, Sambalpur, Bolangir, Jharsuguda); Badshahbhog (Bolangir, Balasore, Koraput, Bhadrak); Durgabhog (Keonghar, Mayurbhanj, Phulbani); Pimpdibsa (Keonjhar); Mugajai (Phulbani, Koraput); Krishnbhog (Puri); Givindbhog (Cuttack); Chinikamini, Saragdhuli, Padamkesri (Konark, Puri); Karpurakali, Pusimakenda (Neyagarh); Kalikati (Kalahandi); Thakurbhog (Puri0; Karpurakanti, Suragaja, Laxmibias (Bolangir, Sambalpur, Deogarh); Tulsiphulla (Puri); Gangabali (Ganjam); Kanikakala
Punjab (2)	Basmati 370 (Amritsar, Gurdaspur, Jullundur); Quadian basmati (Amritsar, Gurdaspur)

States	Varieties (district)
Rajasthan (6)	Basmati, Danger, Sutar, Pathania, Ratipanne, Zed zeera
Tamil Nadu (1)	Jeerakasambha
Tripura (5)	Govindbhog (white); Govindbhog (black); Sadakhaja, Kalakhau, Kalijira
Uttar Pradesh (20)	Kalanamak (Basti, Sidharthnagar, Maharajganj, Gonda, Goroli); Adamchini (Balia); Bindli (Pauri); Badshahbhog (Bareilly, Rae Bareilly, Allahabad, Partapgarh); Batanphul (Basti, Sidharthnagar, Ajana, Mau, Sultanpur); Benibhog (Barabanki); Dhania (Basti, Gonda); Dulhania (Baraich); Hansraj (Dehradun, Rampur, Pilibhit); Jeerabati (Basti, Varanasi); Kamalijira (Basti, Sidharthnagar,Baraich); Lalmati (Baraich, Barabank); Laungchoor (Mirzapur, Varanasi); Phoolchameli (Varanasi, Mirazapur, Son Bhadra); Ramjawain (Basti, Sidharthnagar); Shakarchini (Varanasi, Mirzapur, Son Bhadra); Ramjawain (Basti, Sidharthnagar);Sakarchini (Varanasi, Mirzapur, Son Bhadra); Sonachur (Mirzapur, Varanasi); Tilakchandan (Rampur, Pilibhit, Nainital); Tulsimanjri (Balia); Vishnuparag (Barabanki)
West Bengal (15)	Radhunipagla (Birbhumi, Bankura, Burdwan); Badshabhog (Burdwan, Hooghly,Bankura); Kalonunia (Doars, Jalpaiguri); Kataribhog, Seetabhog (Dinajpur); Gandheswari (pocket); Chinisakar (Raiganj); Ramtulsi (Darjeeling); Tulsibhog (north Bengal); Tulaipanji (Dinajpur);Mahishadan (Bankura); Govindbhog (Hoogly, Howarh, Nadia); Patina, Basmati, Kalijira

North-East India Scenario

The Indian subcontinent features a wide range of common rice types, which may indicate a significant grain domestication role. The North East Indian territories are Assam, Arunachal Pradesh, Manipur, Mizoram, Meghalaya, Nagaland, Tripura, Sikkim, and Mizoram, which span over 255,000 square kilometers. In NE India, it is estimated that 10,000 indigenous rice cultivars of agronomic, ecological, and cultural significance remain (Hore *et al*., 2005). Such a large rice gene pool may contain a variety of agronomic and ecologically important traits. This area has a diverse variety of locally embraced non-basmati aromatic rice germplasm, in addition to other conventional cultivars, which have enormous cultural and economic significance. However, the rest of them have low productivity and are grown solely for their socio-cultural value (Roy *et al*., 2015).

Rice is grouped into various categories based on its features, with the grain shape and kernel type being the most common. Rice is often classified into long, medium, and short grain types, with long grain rice usually measuring more than 6.2 millimetres (mm), or about three times the width of the grain. Rice is between 2.1 and 2.9 times the width of the medium grain. Finally, the short-grain rice group is less than twice as large. Around the globe, fourteen distinct rice varieties are cultivated and eaten, which are classified into subcategories such as glutinous and glutinous free aromatic rice. Aromatic glutinous free rice comprises long seeds with intense flavors, including jasmine and basmati. Another kind of rice is glutinous rice, also known as sticky rice, which comes in both long and short grains and has a high starch content (Shamimagrimet, 2013).

Aromatic Rice Germplasm

Scented rice germplasm is divided into three categories: basmati rice, jasmine rice, and non-scented rice. They are distinguished by their medium-to-heavy fragrance. For the overall eating quality of rice, not only the level of fragrance in general, but also the presence or absence of certain other significant qualities, such as kernel lengths and widths, after-cooking kernel elongation, the concentration of amylose (AC), the temperature at which gelatinization occurs (GT), and the consistency of the gel and flavour are all determined. Basmati rice cultivars are of low to moderate GT, intermediate AC, and medium gel consistency (GC) and have their origins in India and Pakistan. On the other hand, Thai Jasmine rice has low AC and GT and a smooth gel consistency (Jualiano and Villareal, 1993). Jasmine rice kernel length is marginally longer than that of basmati rice, but the latter seems longer because it is more slender. In both varieties of rice, the L/B ratio is almost the same. The ability to lengthen the Basmati rice group almost doubles its original length after cooking, which is its most distinguishing feature. This aspect does not include other scented rice, and although some of them elongate, they are not as evident in length as Basmati.

Biochemistry of Aroma

The chemical 2-acetyl-1-pyrroline was discovered and found as a key contributor to the popcorn-like aromatic rice scent (Buttery *et al*., 1982 & 83). Researchers examined 114 volatile chemicals in cooked fragrant rice to determine the greatest contributor to scent was 2-Acetyl-1-Pyrroline. By using 13-hydrocarbons, 14-acids, 13-alcohol, 16-aldehydes, 14-ketones, 8-esters, and 5-phenols, the volatile chemicals were categorized. When the grain is

cooked, the characteristics of the sweet fragrance of Basmati rice are shown to imitate the aroma of *Madhuca longifolia* flowers.

Recent investigations have nevertheless demonstrated the existence of four additional compounds: pyrrol, 2-acetyl pyrrole, 1-pyroliin and 6M5OTP, 2AP isomers (6-Methyl-5-Oxo-2, 3, 4, 5-Tetrahydropyridine) has a chemical and genetic significant link to 2AP, which distinguishes non-aromatic rices from aromatic rices (Daygon, *et al*., 2017). An effort was made to map three QTLs that regulate 2AP density. On chromosome 8 with a single major QTL (Lorieux, *et al*., 1996, Chen, *et al*., 2008) as well as two chromosomal minor QTLs on 2 and 12 (Lorieux, *et al*., 1996). Since then, map-based cloning has subsequently found the gene involved in grain flavour (Vanavichit *et al*., 2004, 2005). The gene was mapped to a 4.5 kb genomic tract with 15 exons of the 1512-bp coding region, each containing 15 exons of chromosome 8, which converted into 503 sequences of amino acids in non-aromatic rice varieties. This gene is found to be recessive in all aromatic rice due to two critical mutation events in positions 730 and 732, which result in the depletion of an 8-bp "GATTAGGC" commencing at position 734. Several transcriptional and whole genome expression studies have also shown that Os2AP is over expressed in non-aromatic rice varieties, and that in aromatic rice, the premature stop codon of 753 reduces the complete length of the peptide to 252 amino acids due to its suppressive expression (Bradbury *et al*., 2005; Vanavichit *et al*., 2005). According to the hypothesis, this short, unfinished peptide has been confirmed in some cases to induce nonsense-mediated decay, which is thought to be active in all aromatic rice varieties (Chang *et al*., 2007).

AOV is determined by the number of oxygen components by weight needed in typical circumstances to oxidise 105 parts in the sample, and has been linked to the rice flavour of Basmati. It is a measurement of how much flavour volatiles in rice have been reduced.This importance is greatly influenced by alcohol and carbonyl compounds. The AOV, after six months of storage tests with both ordinary as well as Basmati rice species, decreased from 14 to 8 on average for newly harvested rice. This decrease suggests the development of reducing flavour compounds in old rice (Buttery *et al*., 1982).

Markers of Aroma Genes

Any breeding programme requires a simple assay to monitor inheritance. They added KOH to the plant sample, which emitted the fragrance and produced an assay for measuring

the aroma from plant content (Sood and Siddiq, 1978). The strong scent was diverse in 117 lines, whereas 28 lines had a mild scent (Jin *et al*., 1996). An aroma gene marker was discovered using the RAPD method (Ahn *et al*., 1992). The fragrance gene was tagged using RFLP methods. 2-acetyl-1-pyrroline plays an important part in adding a spice or flavouring to non-aromatic rice in order to impart the fragrant of scented rice (Buttery *et al*., 1985). Additional considerations of cooked rice include grain flavour, size, elongation, whiteness, texture, stickiness, and market acceptability, even though the scent is possibly due to a mixture of different compounds.

In the processing, storing, milling, cooking, and eating areas, aromatic rice emits specific aromas. The growth of the fragrance and aroma is enhanced when aromatic rice is grown in areas with cooler temperatures during maturity. Ricer breeders have employed a range of methods to research the heritage of rice scents to analyse and identify scents, involving chewing a couple of seeds and cooking a seed sample of each plant and noting the fragrance. The characteristic fragrance has also been recorded in the leaf tissue of scented plants (Nagaraju *et al*., 1975; Sood and Siddiq, 1978, 1980). In aromatic rice sampling, the concentration of 2-acetyl-1-pyrroline may be altered by cultural, harvester, and post-harvest activities (Goodwin *et al*., 1994). The disparity between non-aromatic rices and aromatic rices is because of differences in 2-Acetyl-1-Pyrroline contained in grains (Buttery *et al*., 1986 & 1983). As a result, transforming non-aromatic rices into aromatic rices must involve a change rather than a new biochemical pathway.

It was confirmed that a single dominant gene controls rice aroma (Jodon, 1944), while digenic and trigenic (Kadam and Patankar, 1938; Nagaraju, *et al*., 1975; Dhulappanavar, 1976; Reddy and Sathyanarayanaiah, 1998) recorded the presence of four complementary genes in scent regulation, one of which was linked to a complementary gene for apiculus red pigmentation. Two dominant complementary genes, SK 1 and SK 2, were found to regulate aroma (Tripathi and Rao, 1979). The trial verified that the fragrance gene is mapped to chromosome 8 of the rice genome using the RFLP technique. Furthermore, these genes have no control over the genes that regulate the colour of the leaf sheath, the maturing hull, etc. 36 of the 37 marker genes analysed were isolated independently of the scented gene (Ahn *et al*., 1992).

About the Experimental Plant

For more than half of mankind's life is rice (*O. sativa* L.). Every third human on the planet consumes rice in some way or another every day. The rice plant is a kind of grass that produces rice, an edible grain. The International Rice Research Institute has stored over 1,00,000 rice adhesions (IRRI). The genus Oryza has 2 cultivated and 22 wild species. There are about 1,20,000 different types of rice. The features of species divergence include biotic and non-biotic influences such as productivity, susceptibility to disease and insects, cold and drought tolerance, and many other variables. *O. sativa* (2n = 24 AA), usually known as Asian rice, has been grown globally among the two cultivated species, while in West Africa, *O. glaberrima* (2n = 24 AA), or "African rice," is grown in a small area (Shamimagrimet, 2013).

Agronomy

Common traditional aromatic cultivars are tall (160 cm or more), have low grain yields, and may get stuck in heavy nitrogenous fertiliser doses. Blast, stem borer, bacterial leaf blight, and white-backed hopper are all problems that can affect these rice cultivars (Siddiq *et al*., 1997). The seedlings are manually transplanted in the first week of June into waterlogged regions and surpassed when the height of the planting is around 8 inches. This is crucial in maintaining a high return and higher quality. In late October and November, rice is harvested. Large varieties are photosensitive and require a limited flowering induction time. When the day length decreases and a crucial phase for the flowering induction hits sensitive variations by shortening the day, this impact on the blooming affects the time of maturation. Conventional Basmati lines, like Pusa Basmati 1 and Haryana Basmati 1, have a greater photosensitive index/phase than upgraded or recently released Basmati kinds (Ahuja *et al*., 1995).

Aromatic cooked rice efficiency depends greatly on environmental aspects such as soil fertility, irrigation and spacing, transplanting time, harvesting time, and storage (Singh & Singh, 1997). Rice cultivated in alkaline, inadequate soil or with insufficient water supply has inordinate abdominal whiteness ingrains and poor cooking quality, especially during the grain development stage. Early transplantation deteriorates the quality of the cooking because the grains are excessively opaque or because of the inappropriate growth of the starch molecules due to the loss of packaging at high temperatures (Ali *et al*., 1991; Azeez and Shafi, 1996).

Plant Growth Substances

Plant hormones have a wide range of effects on plant development. In both plant morphology and physiology, the growth regulators are critical in their specific actions, depending on the material concentration and the organ sensitivity. The effect of the growth regulator depends on the variety, growth, chemical concentration, method, and frequency of plant species (Hilli *et al*., 2010). Regulatory plant growth are chemicals that cause rapid changes in plant phenotypes as well as affect plant growth when used in limited quantities, whether by enhancement or stimulation of the regulatory mechanism for natural growth, from seed germination to senescence. Diverse chemical combinations have a substantial influence on rice agriculture, morphology, and biological features, and it has been revealed that Plant Growth Regulators (PGRs) produce rapid cell division at low concentrations, resulting in quicker vegetative and reproductive growth, and that they can improve physiological efficiency, including photosynthetic capacity, and enhance effective portioning of accumulation (Kim *et al*., 2006; Amanullah, *et al*., 2010; Kar *et al*., 2011).

A plant growth inhibitor or retardant is another form of phytohormone based on its action. In plants, these chemicals hinder growth and foster dormancy and abscission. Plant growth retardants are most often involved in extending or elongating the cells, where Gibberellin synthesis inhibition quickly reduces stem elongation and leaf expansion (Tanomoto, 1987; Leclerc, *et al*., 2006) and reduces cell division and cell elongation (Rademachar, 1991, 1993, & 2000; Boldt, 2008). The number of lateral shots increases with growth retardants, which leads to greater inflorescence (Whealy *et al*., 1988; Keever and Foster, 1989). Various growth retardants decrease the internodal length and the plants are known to reduce their height. As a result, they are often used in the floricultural industry for height management (Bailey and Whipker, 1998; Pasian, 1999; Hayashi *et al*., 2001; Karlovic *et al*., 2004). As a result, the source-sink interaction is influenced, and photosynthesis is stimulated to move from the source to the sink. As a result, the source-sink interaction is influenced, and photosynthesis is stimulated to move from the source to the sink. Growth retardants can also increase the chlorophyll content of leaves, extending the source's functional life and improving portioning quality and productivity (Kanp *et al*., 2021; Pati, 2019; Ojha, 2014; Kashid, *et al*., 2010).

Accelerate aging

The accelerated test was performed to quantify the stored seed and assess the force involving the sensitivity of the seed to unfavourable temperatures and 100% R.H. for different times, followed by routine tests of germination (Delouche and Baskin, 1973). Because of the high temperature and humid atmosphere, the seeds absorbed moisture and aged quickly. The basis of this test is that high-vigor seeds can withstand the high-temperature, high-moisture treatment and to maintain their capacity in germination testing to generate average seedlings. Many crops have suggested and recommended accelerated ageing tests, and some trials have been conducted in **rice seeds** (Henge, *et al*., 2019; Baek, *et al*., 2018; Ali, *et al*., 2003; Krisnasamy and Seshu, 1990), **wheat seeds** (Bhattacharyya *et al*., 1985), **French bean seeds** (Pandey, 1989), **cotton seeds** (Basra *et al*., 2003), **carrot seeds** (Al-Maskri, *et al*., 2003), **aubergine, cucumber and melon seeds** (Demir, *et al*., 2004), **beet root** (Silva, *et al*., 2006), **kale seeds** (Komba, *et al.,* 2006), **soybean seeds** (Torres, *et al*., 2004), **sunflower seeds** (Pati, *et al*.,2012; Vijay Kumar, 2015; and Kanp, *et al*., 2021), **black gramme seeds** (Pati, *et al*.,2019), **Mungbean seeds** (Bhattacharjee, *et al*., 2006; Luciana, *et al*., 2019), **corn seeds** (Dutra, *et al*., 2004; Pati, *et al*., 2014) **Radish seeds** (Jain, *et al*., 2006) **Onion seeds (** Jangjoo, *et al*., 2020) have also proved to be effective for an accelerated ageing test as a seed vigour test.

In the warehouse store, accelerated ageing was originally utilised to evaluate the vitality of seeds (Delouche 1965, quoted in AOSA 1983). Seeds that are stored well after accelerated ageing have a high survival rate, while seeds that have a lower germination rate after accelerated ageing have a rapid storage decline. Under a lot of circumstances following accelerated ageing (Delouche and Baskin, 1973), germinating responses were strongly associated with storage responses under a lot of circumstances. The consistency of the mung beans and the maize seed in the bags of paper indicated that the best longevity assessment in the humid tropics was accelerated by 43ºC for 96 hours and 44ºC for 96 hours, respectively (Santipracha, *et al*., 1993). According to another study, the easiest way to distinguish mungbean seed lots is to use a combination of 42ºC temperature and 72 hours of seed exposure for the accelerated ageing test (Luciana *et al*., 2019). A combination of 45ºC/72 h conditioning of the seeds in only one layer for corn and 42ºC/48 h conditioning of the seeds in only one layer for soybean was the most effective process for separating the lots in terms of vigour (Dutra *et al*., 2004).

Principles of accelerate aging test

The accelerated ageing test significantly improved seed degradation by exposing it to elevated ambient temperatures between 40°C and 45°C and high relative humidity above 90% for short intervals of 48 hours or longer based on species (AOSA, 1983). The loss of physiological potential as a result of accelerated ageing is commensurate with the physiological potential of the seeds. High-vigor seeds showed moderate declines in germination after accelerated age treatment, but low-vigor seeds showed significant declines. Furthermore, after accelerated aging, the germination response of seed lots was compared to field performance under a wide range of conditions (Delouche and Baskin, 1973).

Accelerated aging as seed vigor test

Delouche invented accelerated ageing as a seed consistency test in 1965. The method for forecasting seed viability during storage was initially created in the warehouse (AOSA, 1983; Khan *et al*., 2017). The following investigations have proven the exactness of the test to determine the life cycle of various seed types under different storage circumstances (Delouche and Baskin, 1973). The accelerated ageing test was proposed by Baskin in 1970 and would have additional usefulness for the prediction of seed production beyond storability, with the aim of predicting the establishment of peanuts. Other investigations have now demonstrated the equivalent efficacy of the accelerated ageing test in the prediction of seed establishment from diverse crops.

Benefits of accelerating ageing test

According to surveys of seed test facilities in North America, the accelerated ageing test is among the most commonly used viability tests in laboratories for seed research. It is quick, easy, and economical; no sophisticated facilities are necessary and it can be done without training by anyone. Seed vigour tests may also be used for the assessment of future plant seed storage in the prediction of field outcomes (TeKrony, 1993; Ferguson, 1990; AOSA, 1995). Seed vigour testing has been recommended for accelerated ageing tests in different crops under common conditions. The accelerated ageing process was effectively connected and demonstrated as a signal of the seed viability in the emergence and stand establishment of a large variety of crop species (AOSA 1983 and ISTA 1995).

A number of studies of the findings from the accelerated ageing test in crop seeds showed that accelerated ageing tests would forecast the development of fields such as **wheat seeds by** (Tomer and Maguire, 1990), **sweet corn** seeds by (Singhabumrung and Juntakol, 2004), **soybean** seeds by (Egli and TeKrony, 1995; Torres, *et al*., 2004; Patil, *et al*., 2018), **cotton seeds by** (Bishnoi and Delouche, 1980), **watermelon** seeds by (Mavi and Demir, 2007), **pepper** seeds by (Sundstrom, *et al*., 1986), **pea** seeds by (Kanp, *et al*., 2009) and **rice** seeds by (Chea, 2006; Kapoor, *et al*., 2011; Baek, *et al*., 2018; Henga, *et al*., 2019).

Accelerated ageing is a physiological resistance test that allows regulated seed degradation since exposure is above 90% at high temperatures and high relative humidity (ISTA, 1995; Begnami and Cortelazzo, 1996). Relative humidity, temperature, and humidity and their effects are essential factors that affect the life cycle of seeds. Most crop seeds are less viable at 80 percent relative humidity and temperatures ranging from 25 to 30^{o} Celsius (Copeland and McDonald, 1995).Rice seed ageing is linked to the content of seed humidity and high temperatures, which affect the metabolism of the seeds. Higher temperatures improve the pace at which certain enzymatic and metabolic reactions occur, increasing the metabolic activities of hydrolyzed substrates and enzymes, resulting in a faster rate of degradation, whereas high relative humidity raises the moisture content of seeds, resulting in biochemical events such as increased hydrolytic enzyme activity and free fatty acids (Copeland and McDonald, 1995 & 2001).

Since seeds were exposed to an accelerated ageing test, some researchers looked at how they deteriorated. Many times, the deleterious modifications that occur throughout the process of ageing have been discussed in depth (Delouche and Baskin, 1973). The first symptom of seed decay was cell membrane depletion, which was caused by oxidation in the phospholipid membrane by chains of fatty acid (McDonald, 1999). Increasing solute leakage leads to a decrease in cell membrane integrity, which is the first direct expression of degradation (AOSA, 1983). Sub-cellular organisation was disrupted, enzyme activity was reduced, respiration rate and performance were reduced, and total macromolecule synthesis was reduced, according to electron microscope tests. Before the reduction in germination population was found in the root of aged seeds, degraded DNA and an increase in 32 chromosome abnormalities were suspected (Powell, 2006). Membrane degradation of soybean seeds was the first symptom of seed decay after increased ageing by lipid peroxidation (Panratsamee, 2008). Due to accelerated ageing, the decay of seed in the

embryonic axis of a peanut has triggered changes in the integrity of the membrane, resulting in seed leaks of essential electrolytes (Arguello, 1995).

Protein synthesis rate reduction in radish under accelerated ageing conditions can be attributed to a decrease in protein synthesis, an increase in proteinase degradation activity, or a combination of the two (Jain *et al*., 2006).There have also been reductions in total protein content in pigeon peas due to decreased protein production as well as a steady depletion of seed viability (Madhava Roo and Kalpana, 1994), **sunflower** (Pati, *et al*., 2012), **and maize** (Bhattacharjee, *et al*., 2014). Under accelerated ageing conditions, seeds lose viability at a rapid rate. Even cotton has been observed with low viability, vigour, lipoxygenase activity, acid phosphatase activity, and lipid content (Freitas *et al*., 2006). Henga *et al*. (2019) have discovered that extended exposure to higher temperatures reduces rice seed viability. Instead of causing stress, higher temperatures will facilitate the denaturation of proteins and the death of seeds.

Chemicals used in this Investigation

Sodium dikegulac (NaDK)

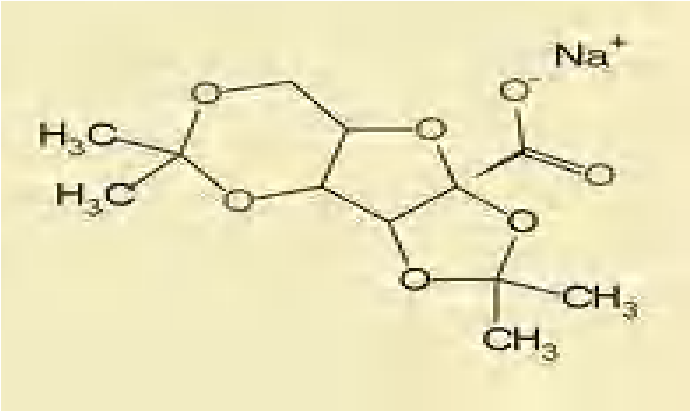
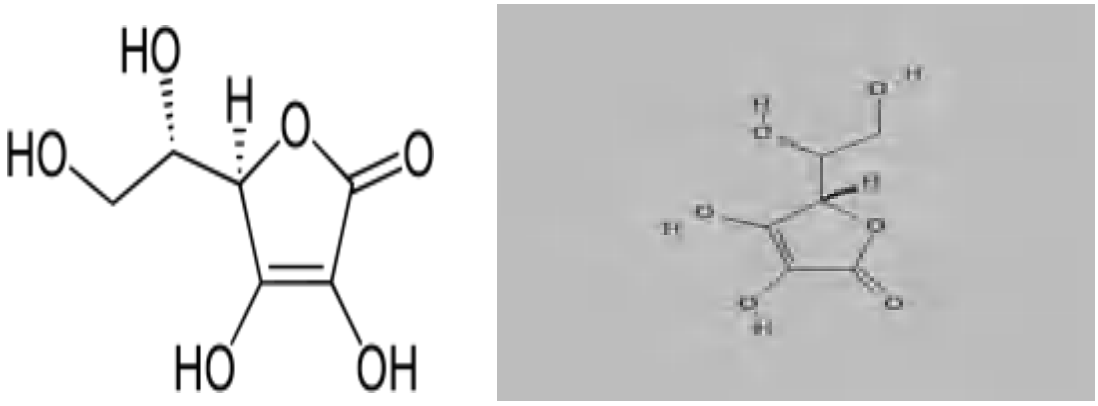


Fig.1. NaDK (Formula $C_{12}H_{17}NaO_7$ & Molecular weight 296.25)

Sodium dikegulac, commercially named as Atrinal, has been discovered as an influential growth retardant with a molecular formula of 2,3:4-6-Di-0-Isopropylidene-Lxylo-2-Hexalofuranosate. It is white, odourless, solid, and photosensitive. In water, methanol, and ethanol, Na-Dikegulac is very soluble but not so soluble with chloroform, acetone, cyclexane, and hexane. In liquid solutions, it is stable at pH 7 or above.

A large number of plant progressions, metabolic activity, production, usefulness, and other characteristics of a diverse range of plant species have been studied in various ways (Bocion, *et al*., 1975; Arzee, *et al*., 1977; Zilkah & Gresel, 1980; Purohit, 1980; Bhattacharjee, *et al*., 1984, Chhetri, *et al*., 1993; Bhar, 2011; Bhattacharjee, *et al*., 2014; Lama, *et al*., 2016; Pati, *et al*., 2018; Kanp, *et al*., 2021).NaDK is a monosaccharide-based sucrose hormone, with several salts used as intermediates in the commercial manufacture of L-ascorbic acid and sodium-connected to this hormone having been shown to be the most successful in showing hormonal activity. It inhibits gibberellins and auxins, although in the usual sense, it is not anti-gibbrellin or anti-auxin. The substance is relatively low in toxicity and does not irritate the eyes or skin. Agri-horticulturists are particularly interested in the chemical because of its chemical pinching property.

Ascorbic Acid (ASA)



L- ASA D-ASA

Fig.2. L and D -Ascorbic Acid ($C_6H_8O_6$) (Molecular weight 176.12 g/mol)

Hexuronic acid was the original name for ascorbic acid, which has the formula $C_6H_8O_6$. Since it is a white solid, impure samples may look yellow. It readily dissolves in water and produces a slightly acidic solution. It is a mild reducing agent that comes in two enantiomers (mirror-image isomers): "L" (for "levo") and "D" (for "dextro"). The L isomer is the most widely seen, appears naturally in many foods, and is one source ("vitamin a") of vitamin C, an important nutrient for humans and many animals. It is used for its antioxidant

qualities as a food additive and a nutritional supplement. The "D" type is possible by chemical synthesis, but does not have an important biological function.

Succinic acid 2,2-dimethylhydrazide (SADH)

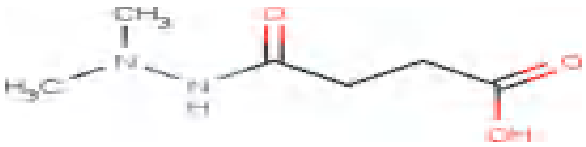


Fig.3. Succinic acid 2, 2-dimethylhydrazide (Formula $C_6H_{12}N_2O_3$ & Molecular weight 160.173).

Succinic acid is a heat-sensitive, scentless white crystal and powder of the molecular formula $C_6H_{12}N_2O_3$, 154-156ºC, melting point, water-soluble. It is also called aminocide and is used as a growth control for a wide range of plants and adornments. Inorganic fluorides, halogenated organics, isocyanates, ketones, metals, amides, carbohydrates, cyanides, or phenols can create hazardous gases, as well as combustible gases, epoxides, acyl halides, and other heavy oxidising and reduction agents. Powerful oxidising agents, metal salts, peroxides, and sulphides can also cause explosive reactions.

SADH, also known as alar, daminozide, kylar, B-995, daminozide, is known to retard plant growth and has been commonly used to monitor plant size and fruit maturation for agricultural and horticultural applications (Rademacher, 2000). Chemicals are sprinkled on fruits to control growth, facilitate harvesting and prevent apples from dropping out of the trees until they mature, storing them in a reddish and solid state. Alar was licenced for use in the United States for the first time in 1963. It was used on apples until 1989, when the company voluntarily stopped using it after the EPA recommended banning it due to fears about cancer threats to customers (USEPA, 2012).

This is a U.S. manufacturer of daminozide, which was registered for use on human fruit in 1963 by Uniroyal Chemical Company, Inc. (now Chemtura Corporation). It was also employed on cherries, peaches, pears, Concord grapes, transplants of tomatoes and peanut

vines, in addition to apples or ornamental trees. Daminozide has an influence on the starting of fruit tree flowers, ripening, hardness, and colouring, decline of pre-harvest, and uniformity in harvesting and storage of fruits. Daminozide usage on U.S. food crops was deemed prohibited by the EPA in 1989, but allowed it on non-food crops such as ornamental plants (EPA 2006).

Maize is a common crop farmed all over the world. Pretreating maize seeds for 8 hours with sodium dikegulac (NaDK) or maleic hydrazide (MH) delayed the aging-induced rapid loss of germination. Furthermore, as compared to age-accelerated seeds that had not been treated, the treated seeds outperformed untreated seeds in terms of germination percentage, field emergence capability, fresh and dried weight of entire plants, and length of root and shoot. Unlike controlled plants, the seeds treated with NaDK and MH were grown with increased protein, chlorophyll, DNA and RNA levels, greater catalase and decreased amylase activity (Pati *et al.,* 2014; Kumar Nandi, *et al*., 2016).

During seed storage, an aqueous solution of ascorbic acid increased rice germination and seedling vigour. The study sought to determine whether priming plant seeds with hormones and vitamins such as growth regulators and ascorbate could aid in the energisation of coarse and fine rice seeds (Basra *et al*., 2006).In aerated ascorbate (vitamin priming) or salicylic acid (hormonal priming) solutions, rice seeds were soaked for 48 hours. Similar hormone and vitamin priming therapies had the same effect on both rice forms. Both priming treatments enhanced vigour when compared with the control, and ascorbic acid, on the other hand, showed the earliest and most consistent germination and emergence (Pati *et al*., 2011).

The dicotyledonous model plant Arabidopsis thaliana has been extensively studied, particularly plant hormones and their role in innate immunity. In monocotyledonous model rice, plant hormones, on the other hand, have just lately been shown to perform conserved and diverse roles in fine-tuning immune responses. Based on the rice pattern of receptor and resistance protein mediated immunity, evidence suggests that salicylic acid plays an essential part in rice basal protection, but that its activity is more likely to rely on the signalling route than on modifying endogenous levels. Jasmonate, which may be implicated in salicylic acid-mediated resistance, aids rice basal immunity against bacterial and fungal invasion. Ethylene can function as a positive or negative resistance modulator depending on the pathogen type and environmental conditions. Brassinosteroid signalling and abscisic acid promote or protect

against pathogenic infection through several infection/colonization techniques. In rice, auxin and gibberellin are believed to be negative regulators of innate immunity. In addition, as a master regulator for the two hormone paths, gibberellin interacts with jasmonate signals through the DELLA protein for rice growth and immunity (Yang *et al*., 2013).

In three rice varieties, Shaheen Basmati, IR-6 and Super Basmati, a pot test has been carried out to investigate in a glass house the ASA (Abscisic acid), BA (Benzyleadenine) and CCC (cycocel) functions in proline formation, growth, ion accumulation and yield. All the treatments resulted in a significant improvement in shoot and root dry weight over salt alone. Under salt stress, both the CCC and the ABA chemically treated plant cultivars showed a considerable decline in the content of Na+ and a rise in flag leaf K+ content. ABA was found to be more successful than BA and CCC at increasing Ca_2+ content in flag leaves and roots of all cultivars. The stimulatory effect of salts on proline aggregation was further enhanced by the ABA and CCC treatments. IR-6 exhibits stronger proline aggregation and a greater leaf area under salt stress than Shaheen Basmati and Super Basmati (Gurmani *et al*., 2006).

Chapter III

Materials and Methods

3. MATERIALS AND METHODS

Plant Materials

In the Darjeeling Hills, aromatic rice (*Oryza sativa* L.) is cultivated organically by default in nature. *Oryza sativa* is a short-lived annual plant in the Gramineae family that dies after producing seeds during the onset of the winter season in November-December. All experiments in the current investigations have been conducted with stable and viable seeds of aromatic rice collected from nearby areas of Darjeeling district. During the initial collection of rice samples, more than 23 aromatic rice varieties were discovered during field visits. At the time of experimental set up, rice seeds from the local farmers (villagers) in the Darjeeling region were collected freshly, each weighing 3–4 kg of the 14 varieties **(Fig.1)**. Physiological and biochemical studies of these rice varieties were carried out in controlled conditions in the natural climate of the Darjeeling Hills. All the laboratory work has been carried out in the Post Graduate Department of Botany, Plant physiology and biochemistry Laboratory, Darjeeling Government College, Darjeeling, West Bengal, and the field work was carried out in the rice cultivation field at Sunsari Busty, Jamuney, Bijanbari, and Dist. Darjeeling.

During the experimental period, the environmental conditions in Darjeeling were as follows: Temperatures range from 2–9°C to 20–35°C, with a relative humidity of 84–93%.

Systematic Position

- ✓ Kingdom: Plantae [Plants]
  - ✓ Subkingdom: Tracheobionta [Vascular plants]
    - ✓ Superdivision: Spermatophyta [Seed plants]
      - ✓ Division: Magnoliophyta [Flowering plants]
        - ✓ Class: Liliopsida [Monocotyledons]
          - ✓ Subclass: Commelinidae
            - ✓ Order: Cyperales
              - ✓ Family: Poaceae [Grass family]
                - ✓ Genus: Oryza L.[rice]
                - ✓ Species: Oryza sativa L. [rice]

Details of seed morphology, external features of 14 varieties of aromatic rice

The harvested rice is known as "paddy". A paddy is a whole rice seed, and one cereal grain includes one kernel of rice. A grain of rice has four main parts: a seed containing an embryonic rice plant, stored food, and a protective coat. The inedible outer covering is the husk (hull), which is made up of two half-shells that are joined together. Each guards one half of the paddy. The husk is made mainly of silica and cellulose. Its weight accounts for roughly 20% of the total grain weight. Underneath the husk, the bran (germ) is concealed. The next layer is a very thin film of bran. The most nutritious part, fibre, vitamin B complexes, protein, minerals, and fat, make up the majority of the composition of bran. Each grain has an embryo at the base that will eventually develop into a new plant. Grain length, width, and thickness vary widely among varieties.

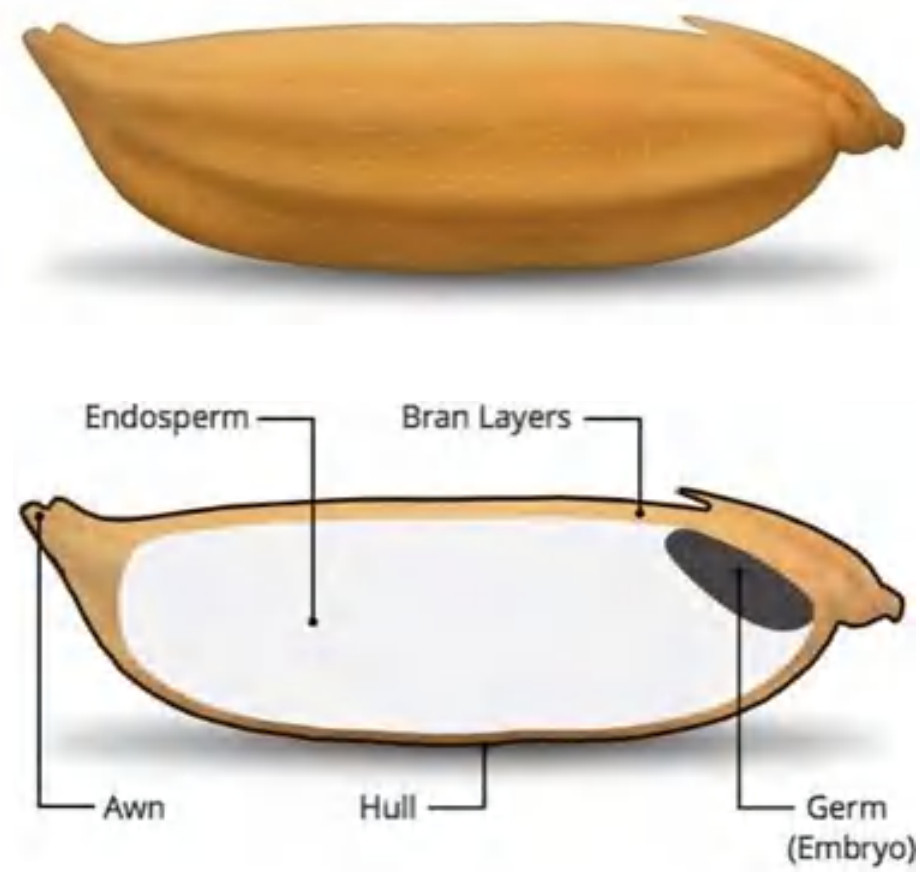


Fig. 4. The Anatomy of a Rice grain.

Three phonological stages of rice plants: (Table 3)

I. VEGETATIVE GROWTH

Stage 0 - GERMINATION - EMERGENCE

Germination and emergence are the first stages in the vegetative phase of growth. Germination begins with the appearance of the young shoot and roots through the seed coat at one end of the seed. The first leaf bursts through the coleoptiles on the second or third day following germination. Stage 0 concludes with the developing main leaf still coiled and an extended radical. **(Fig. 9)**

Stage 1 – SEEDLING

The planting phase begins immediately after emergence and continues until the first tiller appeared. Seminal roots and up to five leaves appear at this stage. Two additional leaves emerge and continue to develop as the seedling grows. Seedling stages occur during the first two to five weeks after planting and are ready for transplanting. **(Fig. 10)**

Stage 2 – TILLERING

This stage continues from the initial tiller appearance to achieve the maximum tiller number. Tillers sprout from the node's axiliary buds and, as they grow and mature, displace the leaves. Once the main tillers have emerged, subordinate tillers are generated. It happens around 30 days following the transplant. The plant is rapidly growing in length and tillering, which develops continually as the plant goes into the next phase, the elongation of stems. **(Fig. 13)**

Stage 3 - STEM ELONGATION

The quantity and height of tillers continue to rise without significant leaf senescence. The length of the stem is proportional to the length of the growth. Longer-growing variants have greater stem elongation. Varieties of rice are divided into 2 groups in this regard: one variety is short-duration (matures in 105 - 120days) and another variety is long-duration (matures in 150days).

II. REPRODUCTIVE GROWTH

Stage 4 - PANICLE INITIATION - BOOTING

Only around ten days after initiation, panicle primordium at the tip of the developing stalk will be apparent to the naked eye. Three leaves will still develop before the panicle appears at this point. The spikelets become distinctive while the panicle continues to grow. The juvenile panicle grows in size, causing the leaf sheath to bulge due to its upward expansion inside the flag leaf sheath, which refers to booting. Almost certainly, the main culm will be the first to fall. **(Fig. 14)**

Stage 5 - FLOWERING

Florets open, anthers emerge from the flower glumes due to stamen elongation, and pollen is discharged as the plant flowers. The florets then shut. It usually opens in the morning. In around 7 days, all of the spikelets in a panicle open. 3 - 5 leaves are still active throughout blooming. **(Fig. 15-18)**

III. GRAIN RIPENING

Stage 6 - MILK GRAIN STAGE

The ripening phase is made up of the last two stages of development, phases 6 and 7. The grain started to be filled with a milky substance at this point. A white, milky liquid fills the grain, which by pressing between the fingers squeezed out milky liquid. The panicle begins to bend and appears green. Senescence is developing at the tiller's base. The two lower leaves, as well as the flag leaf, are all green. **(Fig. 19-21)**

Stage 7 - DOUGH GRAIN STAGE

The milky part of the grain becomes soft dough initially, and firm dough afterwards. The panicle's grains turn from green to yellow. Leaves and tillers are remarkable in their senescence. When the panicle turns yellow, the field begins to look yellowish. **(Fig. 22 & 23)**

Methods of seed analysis

PHASE I

The rice grain quality of 14 aromatic rice varieties (collected for study) was evaluated using freshly harvested healthy rice seeds. Following collection, the seed batches were separated from the husk, and healthy and undamaged seeds were used for experiments. The assessment process was outlined by Cruz and Khush (1989).

EXPERIMENT NO. I

Rice Grain Quality

Rice is usually consumed as a whole grain, milled and cooked cereal. The idea of quality changes dependent on the preparations for which grains must be used, based on the dimension, form, and grain appearance, cooking patterns, softness, flavour, and taste. The desirable characteristics of rice may differ from one ethnic group or geographic location to the next (Juliano *et al*., 1964). The following information was used to assess the quality of rice grains:

Grain size and shape

The selection of large seeds has been a crucial aim during domestication, since grain size is one of the most important components of grain yield. Modern approaches to understanding the molecular and genetic processes of grain size control can increase rice yields. The final and full size of grains in the spikelet hull are maintained by cell proliferation and cell growth, which escalate storage capacity while restricting the filling of grains (Li *et al*., 2018).

In terms of rice quality, grain size, shape, length, breadth, and ratio of kernels (L/B) are all significant. There are short, medium, long, and long-slender types of aromatic rice grains available in all four categories, but the long-slender scented rice types fetch the largest market price worldwide. Based on physical characteristics, rice varieties are classed into two groups: long and short. The rice seed length is the most important factor to consider, and the length-to-width ratio determines the form. The grain can be visually graded based on its size and form. Grain length and form assessment standards vary by country and marketing

location. This approach was largely followed after Kaul (1970). The following is a classification for evaluating grain size and shape:

Grain size classification

SIZE CATEGORY	LENGTH (mm)	SCALE
VERY LONG	MORE THAN 7.50	1
LONG	6.61 - 7.50	3
MEDIUM OR INTERMEDIATE	5.51 - 6.60	5
SHORT LESS THAN OR EQUAL TO	5.50	7

Grain Shape Classification

SHAPE	LENGTH/BREADTH (RATIO)	SCALE
SLENDER	OVER 3.0	1
MEDIUM	2.1 - 3.0	5
BOLD	2.0 OR LESS THAN 2.0	9

Appearance of grains

The rice kernel size and form, as well as chalkiness, translucency, and the grain's "eye," all influence grain appearance. The rice samples are of low retail value and look bad with damaged eyes. In the same way, the higher the chalkiness, the lower the market acceptability. The customer can visually assess the existence or lack of a white belly, a white back, a white centre, a level of translucency and a fracture at the baseline-ventral end of the grain, known as the eye condition. The scales used to assess the endosperm of milled rice chalkiness are as follows:

% AREA WITH CHALKINESS	SCALE
NONE	0
LESS THAN 10%	1
10 TO 20%	5
MORE THAN 20%	9

Rice cooking and consuming characteristics

Many of the rice cooking and consuming qualities of milled rice are influenced by the starch properties, which comprise 90% of it. The starch qualities that influence cooking and consumption aspects are gelatinization temperature and amylose concentrations.

Gelatinization Temperature

10 entire milled kernels with no breaks are selected and placed in plastic containers along with a solution of 10 ml of potassium hydroxide (KOH) 1.7% (0.3035N). All samples are organised in such a way that there is enough room between kernels for spreading. The sealed containers are placed in an oven at 30 °C for 23 hours. On a scale of numerical 7, the starchy endosperm was evaluated visually. Any test includes standard rice type checks with low, moderate, and high gelatinization of endosperm. This approach was largely accepted by Little *et al*. (1958).

Scales for scoring gelatinization temperature are as follows:

SPREADING	ALKALI DIGESTION	GELATINIZATION TEMPERATURE	SCORE
Kernel not affected	LOW	HIGH	1
Swollen Kernel	LOW	HIGH	2
Kernel swollen; collar complete or narrow	Low or intermediate	High- intermediate	3
Kernel swollen; collar complete and wide	Intermediate	Inter-mediate	4
Kernel segregated or split; collar wide and complete	Intermediate	Inter-mediate	5
Kernel merging; dispersed with collar	HIGH	LOW	6
Kernel completely intermingled and dispersed	HIGH	LOW	7

Amylose content

The most significant intrinsic indicator of cooked and processed rice grain behaviour is the amount of amylose in the grain. It ultimately determines the firmness, rice-water ratio, and gloss of cooked rice. On the other hand, rice, which is not waxy or glutinous, is distinguished by moderate amylose, does not solidify, remains moist and tender while cooking, and is preferred. The majority of basmati and non-basmati rice have intermediate amylose (Sood *et al*., 1980). Rice with a high amylase concentration is dry and hard when cooked. These variations highlight the significance of amylose content as selection criteria.

To analyse the composition of amylase in 100mg of rice powder, 9ml of 1N sodium hydroxide and 1ml of 95% ethanol are put into a flask of 100ml. For the starch gelatinization, the ingredients are cooked in a water bath. After 1 hour of cooling, distilled water will be added and the ingredients will be properly mixed together. In a 100ml flask, 1ml of 1N acetic acid and 5ml of starch solution are added. In addition to the distillation water content, 2ml of iodine (2.0g Potassium iodide and 0.2g Iodine in 100ml of aqueous solution) is added and kept for twenty minutes. The absorbance of solutions is calculated at 620nm. The amylose content is calculated and reported using conversion factors in the dry weight result. The readings of optical density (O.D.) from the standard curve generated from potato amylose were compared to get a quantitative estimate. This method was adopted essentially after Juliano (1971). Rice varieties are grouped by Kumar & Khush (1986) on the basis of their amylose content into waxy (0–2%), very low (3–9%), low (10–19%), intermediate (20–25%), and high (> 25%). **(Table 2)**

Grain elongation

Rice grains absorb water while cooking, increasing their length, volume, and width. In high-quality rice, lengthwise elongation without a rise in girth during cooking is preferred. Both genetic and environmental variables, particularly temperature during ripening time, influence kernel elongation. During ripening, an ambient temperature during the day of around 25°C and at night of 21°C has been observed to assist in maximum grain elongation (Sood, 1978).

For elongation testing, 10 whole kernels were measured and immersed for 30 minutes in 20ml of dH_2O. All the samples have been put into a water bath and stored at 98° Celsius for 30 minutes. The rice was cooked and placed on filter paper-lined in Petridis. All cooked

grains are selected and measured. The average length of cooked rice grains compared to raw rice grains is proportionate to the average length of these grains. The method was largely embraced by Azeez and Shafi (1966). **(Fig. 25) (Table 2)**

Evaluation of aroma

1 gram of freshly milled rice is put in a centrifuge tube with 20 ml of dH_2O to test for the presence of aroma. All samples are then placed for 30 minutes in a water bath after the tubing has been wrapped in aluminium foil. The cooked samples must be allowed to cool before analysing the presence of fragrance in each sample. The samples were categorised as non-aromatic, somewhat aromatic, moderately aromatic, and strongly aromatic. For contrast, checking highly scented varieties are utilized. This fundamental laboratory technique was developed at IRRI in 1971. **(Table 2)**

PHASE II

Design of the experiment

Five aromatic rice types, namely Kalonunia, MohanBhog, Khemti, MasinoBasmati, and Musli, were chosen for this study based on their flavour, elongation of kernels, and gelatinization properties, out of fourteen kinds available. These qualities or properties are some of the most significant commercial parameters.

The experiments were designed to investigate the influence of long-term accelerated ageing (0-, 90-, 180-, 270-, and 360-days) on the five local aromatic rice grains chemically processed with Ascorbic acid (ASA), sodium dikegulac (NaDK), and Succinic acid 2, 2-Dimethyl Hydrazide (SADH). Chemicals such as ASA, NaDK, and SADH have been selected following the initial screening test.

Experimental condition and seed treatment

After being surface sterilised for 90 seconds with 0.1% mercuric chloride ($HgCl_2$), all 5 varieties of seeds (250gm) were individually pre-soaked for 6 hours in aqueous solutions of NaDK (1000 and 2000µg/ml), ascorbic acid (250 and 500µg/ml), SADH (150 and 300µg/ml) and dH_2O, then sun dried to their original seed weight with some moisture content. The soaking and drying procedures were performed three times in a 48-hour period, with a

cumulative pre-treatment time of 18 hours. This method of pretreatment allowed maximal chemical infiltration while preventing the start of germination. (Kanp, *et al*., 2021 & 2009; Pati, *et al*., 2020, 2019, 2014, & 1912; Das, *et al.*, 2003; Maity, *et al*., 2000; Bhattacharjee, *et al*., 1986 & 2006)**.**

This experiment was carried out in an artificially induced environment known as accelerated ageing in order to achieve a comparatively uniform and immediate outcome. The pretreated seed lots (250 gm) were placed into separate cloth bags after complete pre-treatment of the seed lots. In total, 35 muslin bags were placed in desiccators in which a relative humidity of 98.2% was pre-imposed by holding 250 ml of 5.96 percent Sulphuric acid (vol/vol) inside them. This experimental set-up allowed forced ageing and regularly (within 10–12 days) changed sulphuric acid periodically in order to restore the required relative moisture during the experimental time. After the imposition of an accelerated ageing condition on seeds in storage, tests were conducted at 90-day intervals (0-, 90-, 180-, 270-, 360-days) up to 360-days. The control group consisted of seeds that had been pre-treated with distilled water before being subjected to accelerated ageing. The techniques for sampling and the methods for analysing the parameters are outlined in considerable detail here.

In accelerated ageing treatments, vigour and viability of seeds are determined (Heydecker, 1972; Priestley, 1986; Chhetri, *et al*., 1993; Copeland and McDonald, 2001; Rai, 2000; Kapoor, *et al.,* 2011; Pati, *et al*., 2017; Patil, *et al.,* 2018; Henga, *et al*., 2019; Lama, *et al*., 2020; Alahakoon, *et al*., 2021). Under adverse conditions, experiments were designed to study such stable physiological and biochemical data in consecutive periods (0-, 90-, 180-, 270-, and 360 days, respectively) under the influence of three growth inhibitors, ASA, NaDK, and SADH.

EXPERIMENT NO. II

Physiological analysis of accelerated/stored seeds (0-, 90-, 180-, 270- and 360-days) of each pretreatment of ASA, SADH and NADK at an interval of 90 days

Analysis on response of seeds towards T_z salt (0.1%)

The viability of seeds is the degree to which the seed is metabolically alive and contains all the enzymes that can catalyse metabolic reactions for germination and seedling growth. To determine TTC-stainability, 5 varieties of aromatic rice seeds (100) were soaked

in 0.1% TTC solution (w/v) (2, 3, 5-Triphenyl Tetrazolium Chloride) in Petri dishes in darkness for 24 hours. The red-colored stained seed percentage is estimated using the total number of seeds for ASA, SADH, and NaDK treatments. This approach was largely introduced by Halder (1981). Data was collected at 90-day intervals starting from (0, 90, 180, 270- and 360 days, respectively). **(Table 1.4) (Fig. 24)**

Analysis of dehydrogenase activity

For dehydrogenase activity testing, 1g of standarised seed from each procedure was immersed in 10ml beakers containing TTC 5ml (0.1%) solution for 48 hours incubated in darkness. The generated atoms of hydrogen by full dehydrogenase activity engaged in the process of respiration convert tetrazolium in live tissues to a chemical known as formazen (red colour) (Moore, 1973). TTC is reduced in the seeds by a group of enzymes known as dehydrogenases. The formazen was extracted after incubation and the solution O.D. values were measured at 520nm. The Rudrapal & Basu (1979) tetrazolium chloride assay was used to evaluate the complete dehydrogenase activity in undamaged seeds. Data was collected at 90-day intervals starting at (0-, 90-, 180-, 270-, and 360-days, respectively). **(Table 1.5)**

Analysis of seed germination

To assess percentage germination, 100 rice seeds from all treatments were separately transferred to Petri plates containing soaked filter paper in 10 ml of distilled water. After 240 hours of seed soaking, germination findings in terms of percentage of seed germination were reported according to the ISTA (International Rules of Seed Testing, 1976). Analyses were performed at 90-day intervals (0-, 90-, 180-, 270-, and 360-days respectively) up to 360-days after seeds were subjected to an accelerated ageing condition in storage. **(Table 1.6)**

EXPERIMENT NO. III

Biochemical analysis of accelerated/stored seeds

Analysis of protein levels in seed kernels

Protein samples were collected from five varieties of aromatic rice seeds from each of the seven treatments and each ageing period (0-, 90-, 180-, 270-, and 360-days). Rice seeds (100 mg) were homogenised in a motar with 80% ethanol and centrifuged at 6000 g for 10

minutes.Following the process of Kar and Mishra (1976), pellets were washed twice with cold Trichloroacetic acid 10% (w/v), once with ethanol, with ethyl alcohol: chloroform once (3:1, v/v), with ethanol: ether once (3:1, v/v), and once with solvent ether finally. After that, the pellets were dried out by evaporation. After 1 hour of digestion at 80^0C with 0.5 (N) NaOH, the protein was isolated from the pellet and was rendered in a specific amount (4 ml). It was then calculated using the Lowry *et al*., (1951) process, which involved Folin Phenol reactions and calculating the O.D. values in a spectrophotometer at 650nm. For quantitative determination, the O.D. values were compared to a previously constructed standard curve based on BSA (Bovine Serum Albumin, Fractin-V-Powder, Sigma Chemical Company, USA). Data was collected at 90-day intervals starting at (0-, 90-, 180-, 270-and 360-days, respectively). **(Table 1.7)**

Analysis of soluble and insoluble carbohydrate in seed kernels.

Carbohydrate levels (soluble and insoluble fractions) were calculated using the McCready *et al*. (1950) approach with minor modifications. Every 10 minutes, 100mg of 5 different aged seed samples are homogenised at 6000g with boiling 80% ethanol. The supernatant was put in a watch glass. This was done three times, and the surface of the watch glass was placed in test tubes after being washed multiple times with 80% methanol and having the amount increased to 10 ml. This has been retained as a soluble carbohydrate source. **(Table 2.9)**

The residue following centrifugation of the sample was put in a water bath for 30 minutes with 5 ml of 25% H_2SO_4 at 80°C for the analysis of insoluble carbohydrates. The substance was removed as a source of insoluble carbohydrates after adequate dilution. **(Table 2.10)**

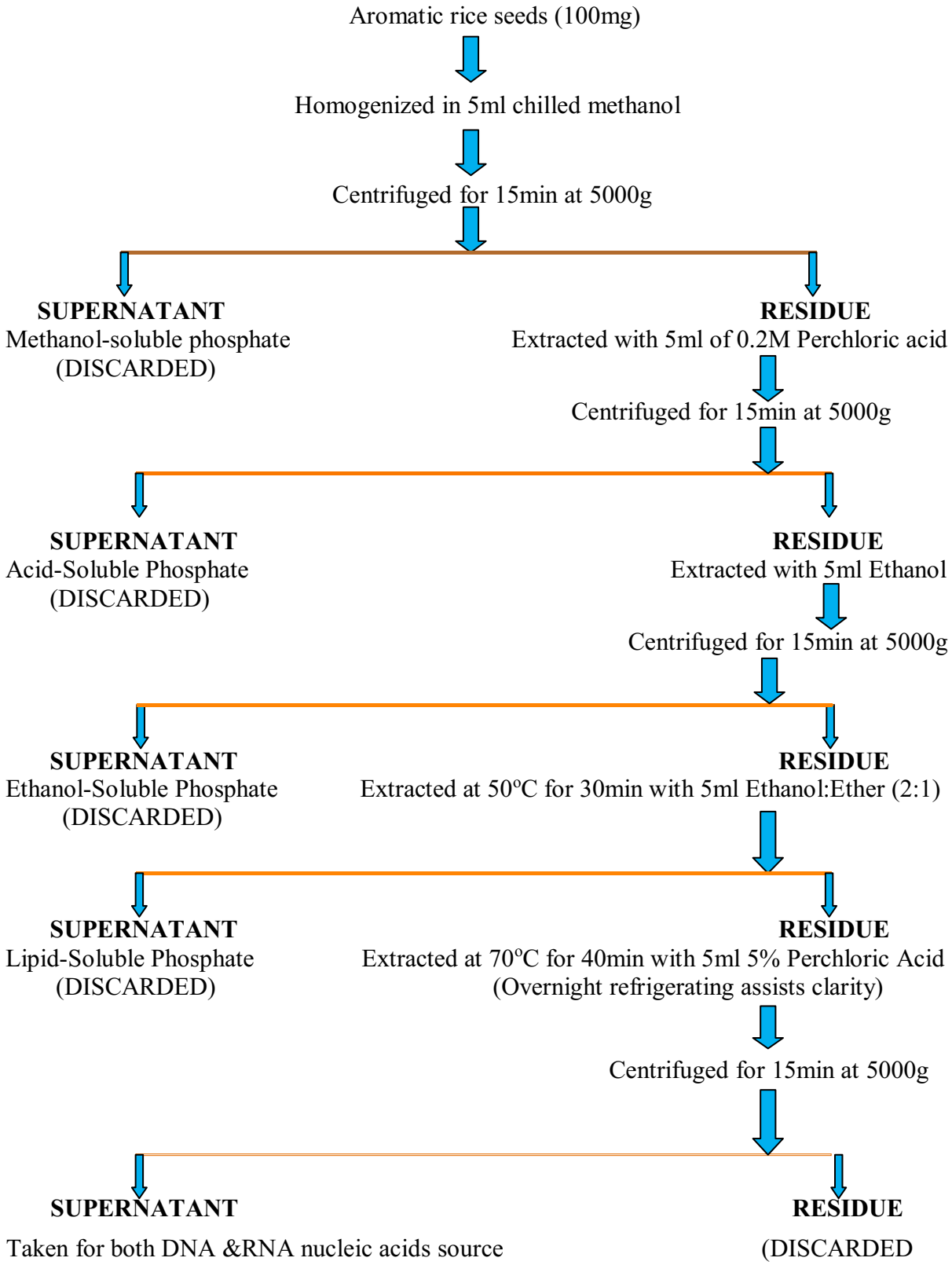
One millilitre of the source sample from each carbohydrate fraction was placed in a test tube, and four millilitres of freshly processed, pre-cooled, 0.2% anthrone reagent (200 mg of anthrone in 10ml of analar H_2SO_4) were applied to it for analysis and quantitative calculation. The spectrophotometer at 620 nm was used to calculate the strength of the green colour after 30 minutes. The actual contents were calculated using a regular curve made with glucose solution concentrations. Data was collected at 90-day intervals starting at (0-, 90-, 180-, 270-, and 360-days). **(Table 2.9 & 2.10)**

Analysis of free amino acids from seeds kernels

Using a clean, pre-chilled mortar and pestle, 100mg of rice seeds from various pre-treated cultivars were placed. The disorganised sample was centrifuged for 10 minutes at 5000rpm after homogenising the seeds of each (7) procedure and each (0-, 90-, 180-, 270-, and 360-day) ageing state with 5ml of 80 percent ethanol.The supernatant was then used as a source of free amino acid content by Dwivedi *et al*. (1979). Amino acid has been estimated using the Moore & Stein process from the stock solution (1948). For 15 minutes, a test tube containing 1 ml of 0.3% ninhydrin solution (80% ethanol) and marbles on the test tube tops were placed in a water bath. After the reaction had cooled to violet, the test tubes were removed and the volume was increased to 5 ml with 80 percent ethanol.A spectrophotometer was used to determine the solution's absorption at 570 nm. To get a quantitative estimate, the standard curve was constructed using glycine as the reference amino acid and compared. Data was collected at 90-day intervals beginning at (0-, 90-, 180-, 270-, and 360-days). **(Table 1.8)**

Analysis of nucleic acid levels from seeds kernels

Nucleic acid extraction (DNA and RNA) was performed on 100mg of kernel seed according to the Cherry method (1962). The DNA as well as RNA estimates were analysed by a standard stock, in which the sample was eventually collected using the Markham (1955) process, updated by Choudhuri and Chatterjee (1970) with perchloric acid (5%). The procedures for nucleic acid extraction are as follows (DNA and RNA):


Aromatic rice seeds (100mg)
Homogenized in 5ml chilled methanol
Centrifuged for 15min at 5000g
SUPERNATANT
Methanol-soluble phosphate
(DISCARDED)
RESIDUE
Extracted with 5ml of 0.2M Perchloric acid
Centrifuged for 15min at 5000g
SUPERNATANT
Acid-Soluble Phosphate
(DISCARDED)
RESIDUE
Extracted with 5ml Ethanol
Centrifuged for 15min at 5000g
SUPERNATANT
Ethanol-Soluble Phosphate
(DISCARDED)
RESIDUE
Extracted at 50°C for 30min with 5ml Ethanol:Ether (2:1)
SUPERNATANT
Lipid-Soluble Phosphate
(DISCARDED)
RESIDUE
Extracted at 70°C for 40min with 5ml 5% Perchloric Acid
(Overnight refrigerating assists clarity)
Centrifuged for 15min at 5000g
SUPERNATANT
Taken for both DNA &RNA nucleic acids source
RESIDUE
(DISCARDED

1. DNA

In an analysis, the extraction of 1 ml of nucleic acid was diluted with 5 ml in a test tube (100 ml of glacial acetic acid, BDH + 2.7 ml of conc. H_2SO_4 + 1 g of AR grade of diphenylamine) of freshly-prepared diphenylamine reagent. In a water bath, the mixture was boiled for 30 minutes with glass marbles on the test tube top. The blue colour intensity in the spectrophotometer was recorded after cooling in running tap water at 610nm. The O.D. values of the herring sperm DNA standard curve were quantified to the DNA content. Data was collected at 90-day intervals starting at (0-, 90-, 180-, 270-, and 360-days). **(Table 2.12)**

2. RNA

In order to estimate RNA, 3ml of freshly prepared Orcinol-reactive reactants (1gm AR dissolved Orcinol in con. HCl of 100ml containing 100mm 0.1 percent $FeCl_3$ and $6H_2O$) were treated with 3ml each of 5 different seed kernel treatments and each ageing period of diluted nucleic acid extract in separate test tubes for 20 minutes in a water bath with marbles on the test tubes' top. The cooled mixture was taken and the spectrophotometer was used to test the strength of the blue-green colour at 700nm. The blank was a combination of 3ml of distilled water and 3ml of orcinol, which were handled identically. The O.D. values from a regular curve made with yeast RNA were used to measure RNA level at 90-day intervals. Data was collected beginning at (0-, 90-, 180-, 270-, and 360-days). **(Table 2.11)**

Determination of the activities of enzyme like catalase from seeds

For each treatment and ageing cycle, a homogenization of 500mg of seed kernels was obtained in 8ml of cold phosphate (Na_2HPO_4/NaH_2PO_4) buffer (pH 6.8). This homogeneous product has been centrifuged in the cold for 15 minutes at 3000g and then for 20 minutes at 10,000g. Using the same buffer, the supernatant was raised to 10 ml and analysed using the Snell and Snell (1971) method, which was revised by Choudhuri and Biswas in 1978. In the reaction of the catalase mixture, add 1 ml of H_2O_2 (0.05M) and incubate for 5 minutes at 37°C. The reaction was stopped by centrifuging the mixture for 15 minutes at 6000g with 0.1 percent titanium sulphate (2ml) in 25% H_2SO_4 (v/v).At 420nm, the golden yellow colour intensity was measured. The sample of blank was usually prepared with the addition of

titanium sulphate by inactivating (heat-killing) enzymes. Data has been collected at 90-day intervals beginning at (0-, 90-, 180-, 270-, and 360-days). **(Table 2.13)**

Determination of the activities of enzyme like IAA-oxidase from seeds

This enzyme was extracted using 100mg of seed samples and 12ml of sodium phosphate (pH 6.1) buffer. At 10,000g, the homogenous product has been centrifuged for 15 minutes. The enzyme's rudimentary source was obtained from the supernatant. The method of Gordon and Weber (1951), modified later by Ramadas *et al*. (1968), was used to assess the behaviour of IAA-oxidase. The reaction mixture contains 1ml of 1mM 2, 4-dichlorophenol, 1ml of 1mM $MnCl_2$, 6ml of 0.03M sodium citrate (pH 4.5) buffer, and 1ml of enzyme extract.The process was halted after 1 hour of room temperature incubation by adding 1 mL of 20% $HClO_4$ to the reaction liquid. In a spectrophotometer, 1ml of the test mixture and 3 ml of Salkowski reagent (50ml of 35% $HClO_4$ + 1ml of 0.5 N $FeCl_3$) were combined, and the results were obtained at 525nm. Data was collected at 90-day intervals starting at (0-, 90-, 180-, 270-, and 360-days, respectively). **(Table 2.15)**

Analysis of enzyme alpha amylase in seeds

The seed kernels of each sample (100mg) were homogenised in 8ml of 0.1M phosphate (pH 6.5) buffer. The homogenate at 5000g was centrifuged for 15 minutes. For the analysis, the supernatant of the enzyme as a crude source was utilized. After mixing 2 ml of enzyme solution with a 0.2 percent starch solution, the mixture was incubated for 30 minutes at 37ºC.To stop the reaction, a 3ml of iodine-HCl solution was employed (60mg of KI and 6mg of iodine dissolved in 100ml of 0.1N HCl). In Biswas and Choudhuri's 1978 technique, the intensity of blue colour was calculated in a spectrophotometer at 610nm. Data was collected at 90-day intervals starting at (0-, 90-, 180-, 270-, and 360- days, respectively). **(Table 2.16)**

Analysis of catabolic enzyme protease in seeds

This enzyme had the same extraction process as the catalase one, with the exception that the used solution was buffered with 6.5pH. The protease activities were determined by incubating 1ml of enzyme extract, 0.1ml of 0.1 M $MgSO_4$, and 1ml of BSA (0.5mg/ml in dH_2O) at 37ºC for 1 hour, then adding 1ml of 50 percent trichloroacetic acid (TCA), and

analysing residual protein with the reagent Folin-phenol (Lowry, *et al*., 1951). This test was done in accordance with the updated Biswas and Choudhuri system (1978). The activities of all assayed enzymes were calculated as [(ATv)/(t v) g wt. of samples], where the O.D. value of A is the blank O.D. minus the O.D. of samples, Tv is the total volume of the filtrate, t (hour) is the incubation time with the substrate, and v is the filtrate volume taken for incubation after Fick & Qualset, 1975.Data was gathered at 90-day intervals beginning at (0-, 90-, 180-, 270-, and 360-days). **(Table 2.14)**

Determination of the activities of enzyme like superoxide dismutase from seeds

To determine superoxide dismutase (SOD) activity, 200mg of seed kernals were crushed in 5ml of 0.1M phosphate buffer (pH7.8) containing 0.1 percent (W/V) insoluble PVPP (polyvinyl polypyrolidene) and at 4^{o}C centrifuged. As a source of an enzyme, the supernatant was employed. The enzyme capacity of nitro blue tetrazolium (NBT) was determined to block the reaction of photochemicals using a modified Giannopolitis and Ries (1977) technique (Roychowdhury and Choudhuri, 1985). The remaining components added to the 3ml reaction mixture were 0.05M Na_2CO_3, 0.1M EDTA, 63M NBT, 13M methionine, 20M enzyme extract, and 1.3M riboflavin. At a distance of 30 cm and at a temperature of 25^{o}C, the test tubes were mounted under two 4W florescent lamps. The light was turned off after 15 minutes, and the absorbance at 560nm was measured. Due to the maximum reduction in NBT, the non-irradiated sample would develop the most colour. The enzyme function was inversely proportional to the reduction of NBT. As a result, A560 of the particular set was subtracted from A560 of the blank set to obtain A (without enzyme). Data was collected at 90-day intervals starting at (0-, 90-, 180-, 270-, and 360-days). **(Table 2.17)**

EXPERIMENT NO. IV

After 360 days of pretreated seeds, seeds needed to be sown in order to find the field emergence capacities of various seed lots. All of the field experiments for this study were conducted in a rice field in Sunsari, lower Goke Busty, Jamuney, Darjeeling, at latitude 27.0951N, longitude 88.2301E, and altitude of 546.22m.

Soil Preparation and method of seeds sowing and transplanting

The field was ploughed 1-2 days before rice seeds were sown. The method is used in places with fertile soil, adequate rainfall, and a large supply of labour. Seeds from each treatment were manually scattered on the prepared flooded area **(Figs. 6, 7 & 8)**. Then water was drained out along with the floated non-viable seeds from the field. Normally, seeds germinate in 3–4 days, but since these seeds were chemically pretreated and stored in forced-accelerated conditions for 360 days, it took more than 10-15 days for them to germinate and seedlings to be prepared **(Fig. 9)**. Usually, seedlings that reached 3-4 inches in height were uprooted and planted on a damp puddled field that had been prepared for the purpose after 21–25 days **(Fig.12)**. Before transplanting, the area should be thoroughly puddled with bullock, which is a critical process. Puddling kills weeds and buries them in puddled soil. It also prevents weed germination during the crop's subsequent growing season. Puddling evens out the soil surface and creates favourable physical, biological, and chemical conditions for rice plant development. **(Fig.7)**

After 25 days of rapid growth and production, the seedlings were uprooted. The entire procedure was carried out by hand. As a result, it is a very complicated approach that necessitates a lot of input. However, it produces some of the highest yields. The winters in Darjeeling are too cold for rice cultivation, so the grain is only grown once a year. Data was obtained from the fields of physiological and biochemical studies.

Physiological field analysis of plant develop from pretreated accelerated/ stored seeds

Recording of field emergence capacity

Seeds were sown in well-prepared flooded soil mixed with a limited amount of cow dung to determine field emergence capability. The readings began the day after seed germination and lasted until the plants entered their senescence level.

Plant growth analysis

For the purpose of studying plant growth attributes, reading began shortly after seed germination and persisted until the plants entered their senescence period. Plant height,

internodal distance, and stem circumference were among the physiological parameters measured. For the purposes of documenting the growth data, the mean values of five plants were used. In case of plant height data were collected before shifting of rice plants in (0-5, 15- and 25- days) and after shifting of rice plants in (45-, 60-, 75-, 90-, 105-, 120- and 135-days) of plant age respectively. Each plant's first internode was used to measure the circumference of the stem. The distance between internodes was used to calculate internode elongation. **(See Tables 3.28 and 3.29)**

Biochemical field analysis of plant leaves develop from pretreated accelerated/ stored seeds

Biochemical analysis was performed for the five rice-plant stages, pre-flowering stage (P), Flowering stage (F), seed formation stage (S), seed mature stage (M), and pre-harvesting stage (H) corresponding to 67d, 86d, 105d, 120d and 132d respectively, taking samples from the leaves of each pretreated plant.

Analysis of macromolecules like DNA And RNA from leaves

Methods have already been described for extracting and estimating DNA and RNA. The samples and data were collected from the leaves of plants that had been grown from accelerated seeds at five stages of growth. The pre-flowering (P), flowering (F), seed formation (S), seed mature (M), and pre-harvesting (s) stages correspond to plant ages of 67d, 86d, 105d, 120d, and 132d, respectively.**(Tables 3.19 and 3.18.)**

Analysis of macromolecules like insoluble and soluble carbohydrates from leaves

Methods of extraction and estimation of insoluble and soluble carbohydrates have already been described. Here, the leaf samples were taken from plants raised from accelerated or aged seeds at four developmental stages. Data was recorded in five phases. Pre-flowering (P), flowering (F), seed formation (S), seed maturation (M), and pre-harvesting (H) stages **(Tables 3.21 and 3.20.)**

Analysis of some scavenging enzymes like catalase from leaves

200gm of leaf tissue from all treatments was homogenised in 8 ml of cold 0.1M phosphate buffer (Na_2HPO_4/NaH_2PO_4) (pH 6.5). The homogenate was, in cool circumstances, centrifuged at 3000g for 15 minutes and then 10000g for 20 minutes. The supernatant was diluted with the same buffer to a level of 10 ml and used as a source of crude enzyme. The activity of the enzyme was determined using the Snell and Snell (1971) technique, modified by Biswas and Choudhuri (1978). 1ml of the aforementioned extract and 2ml of 0.05M H_2O_2 were combined in a catalase reaction mixture and incubated at 37^0C for 2 minutes. The reaction was stopped by centrifuging the mixture for 15 minutes at 6000g with 2ml of 0.1% Titanium Sulphate in 25% H_2SO_4 (v/v).At 420 nm, a golden yellow colour intensity was observed. Prior to the addition of H_2O_2, the blank was prepared with the addition of titanium sulphate by inactivating (heat killing) the enzyme. Data was recorded in five phases, taking leaf samples from each pretreatment plant at the pre-flowering stage (P), flowering stage (F), seed formation stage (S), and seed maturity stage (M). **(Table 3.22)**

Analysis of some Scavenging Enzymes like Superoxide Dismutase from Leaves

Methods of extraction and estimation of enzymes of superoxide dismutase from leaves of plants raised from accelerated or aged seeds at four developmental stages have already been described. Data was recorded in five phases. Preflowering stage (P), flowering stage (F), seed formation stage (S), and seed mature stage (M) leaf samples were collected from each pretreated plant. **(Table 3.26)**

Determination of the level of some catabolic enzymes like IAA-oxidase from leaves

This enzyme was extracted using 100mg of leaf tissue and 12ml of cold 0.2M sodium phosphate buffer (pH6.1). The homogenous product was centrifuged for 15 minutes at 10,000 g. The enzyme was extracted from the supernatant as a crude source. The IAA-oxidase activity was measured using a technique developed by Gordon & Weber, 1951, which was modified by Ramadas *et al*., 1968. 1ml of 1mM 2, 4-dichlorophenol, 1 ml of 1mM $MnCl_2$, 6ml of sodium citrate 0.03M (pH 4.5) buffer, and an extract of 1ml of enzyme were added to the reaction mixture. The reaction mixtures were halted by adding 1 mL of 20% $HClO_4$ after they had been incubated at room temperature for 1 hour. In a spectrophotometer, 1 ml of reaction mixture was reacted with 3 ml of Salkowski reagent (50 ml of 35% $HClO_4$ + 1 ml of

N $FeCl_3$), and the reading was obtained at 525 nm. Data was recorded at five phases: pre- flowering stage (P), flowering stage (F), seed formation stage (S) and seed mature stage (M), taking leave samples from each pretreated plant. **(Table 3.24)**

Determination of the level of some catabolic enzymes like rnase from leaves

At 0^oC, 100mg of fresh leaves were homogenised in 5ml of 0.1M sodium phosphate buffer (pH6.4) and centrifuged for 20 minutes at 10,000g. The supernatant was used as a crude enzyme source and was diluted up to 10ml using the same buffer solution. The assay was carried out according to Biswas and Choudhuri's technique (1978).

The RNase reaction mixture contained 1ml of enzyme extract and 1ml of yeast RNA (1 mg/ml) diluted in 0.1M sodium phosphate buffer (pH5.7). After 30 minutes of incubation, the reaction was stopped by adding 0.2 mL of 70% perchloric acid at 37°C.The supernatant was combined with 5ml of BSA (0.5 micro/ml) diluted in a buffer of 0.1M sodium acetate (pH 4.0) after centrifugation at 6000g. After a few minutes, the developed turbidity was stabilised with 2ml of 0.1 percent gelatin and measured at 420nm.The activity of this enzyme was measured using the Flick and Qualset principle (1975). Data was recorded in five phases. Preflowering stage (P), flowering stage (F), seed formation stage (S), and seed mature stage (M) leaf samples were collected from each pretreated plant.**(Table 3.25)**

Determination of the level of some catabolic enzymes protease from leaves

This enzyme had the same extraction process as the catalase one, with the exception that the solution used was 6.5pH. The activity of proteases has been determined by incubating reaction mixtures comprising 1 ml of enzyme extract, 0.1 ml of $MgSO_4$, $7H_2O$ and 1 ml of BSA for 1 hour at 37^0C, followed by 1 ml of 50% trichloroacetic (TCA) and by using Folin-phenol protease residual can be determined (Lowry *et al*., 1951). This test was done in accordance with the updated Biswas and Choudhuri system (1978).

In all cases of enzyme assayed, the enzyme activities were expressed as [(ATv)/(t v) g wt. of tissue], where O.D. of A is the value of the blank O.D. minus the sample O.D., total volume of the filtrate Tv, incubation time t (hour) with the substrate, and volume v of filtrate after Fick & Qualset, 1975.Data was collected in five stages. Pre-flowering (P), Flowering

(F), Seed Forming (S), and Seed Mature (M) stages, with leaves collected from each pretreated plant. **(Table 3.23)**

Analysis of yield attributes

The following plant yield attributes were recorded from accelerated aged seeds with five varieties of rice aromatic seeds: total seed weight per panicle and 1000 seed weight per plant. The yield data that was presented was an average of three results. **(Table 3.27)**

Chapter IV

Results

4. RESULTS

Table 2 depicts the generalized varietal variations of Aromatic rice cultivate in various altitudinal locations of Darjeeling hill. During the research, a total of 14 varieties of local aromatic rice were discovered, each of which was identified based on the physical and chemical characteristics of mature rice grain.

Important phonological events were shown in **Table 3** during the life cycle of the rice samples.

In particular, out of 14 varieties, the value of food and commercial essence have been used as experimental seed samples for 5 varieties (**Mohanbhog, Khemti, MasinoBasmati, Musli, and Kalonunia)**, and the investigation was conducted after observation.

PHASE: I

Analysis of rice grain quality of 14 different aromatic rice seed varieties (Table 2)

Rice grain size and shape were used to classify 14 distinct aromatic rice seed varieties based on two physical characteristics, length and shape. Five varieties (**Mohanbhog, Khemti, MasinoBasmati, Musli,** and **Kalonunia**) had long to very long grain sizes and were slender to medium in shape, according to the size and shape scale of rice grains. Just two varieties (**Khemti and MasinoBasmati**) exhibited endosperm chalkiness in milled rice, out of a total of 14 varieties. The proportionate elongation was highest in **Musli**, followed by **Mohanbhog**, **Kalonunia, Masino-Basmati**, and **Khemti**, in that order **(Fig. 25)**. Aroma is a distinguishing feature of aromatic rice, with three varieties (**Mohanbhog, MasinoBasmati, and Kalonunia**) being especially aromatic. Varieties with high gelatinization temperatures were recorded in **Mohanbhog, MasinoBasmati**, and **Krishnabhog**, and varieties with low gelatinization temperatures were in **Kemti** and **Musli**. Amylose content was found to be limited in almost all rice types.

PHASE: II

Effect of long-term accelerated ageing (0-, 90-, 180-, 270- and 360- days) of seeds of aromatic rice types, Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia, pretreated with Ascorbic Acid (ASA, 250 and 500µg/ml)), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml)) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml)) on biochemical changes and TTC (2,3,5-triphenyl tetrazolium chloride) stainability of seeds.

Effect of long-term accelerated ageing on seed metabolism

After 0-, 90-, 180-, 270-, and 360-days of rapid ageing, soluble and insoluble carbohydrates, free amino acids, protein, RNA and DNA content, enzyme activity, and total dehydrogenase activity were measured in the seeds of aromatic rice varieties Mohanbhog, Khemti, MasinoBasmati, Musli, and Kalonunia. **(Tables 1.4–3.29)** included the results of forced ageing treatment on modification of seed metabolism after chemical pretreatment with ascorbic acid, succinic acid dehydroxide, and sodium dikegulak. Visual testing has shown that all seed samples have been invaded with storage fungi of different species during long-term accelerated ageing and that the incidence of pathogenic attacks is greatest on controlled (dH_2O) seed lots.

Effect on percentage TTC-stained seeds (Table 1.4)

When results were collected at 0-, 90-, 180-, 270-, and 360-days, the results showed a decrease in TTC stainability in all seed samples with increasing ageing length, regardless of treatment. For Mohanbhog, Khemti, MasinoBasmati, and Musli, the initial percentage of 0-days of TTC-stained pretreated seed samples was over 95 to 100%. In other cases, like the Kalonunia seed sample, the original number of samples of TTC-stained pretreated seed was only 52–65%, and the immediate impact of the pretreated chemical on TTC-stained seed appeared conspicuous. This began to decline as accelerated ageing progressed. When the seed varieties were subjected to prolonged accelerated ageing days of 90-, 180-, 270-, and 360-days, a persistent fall in TTC stainability was observed. This fall was observed to be more extreme and noticeable in control samples of all varieties, but it was significantly reduced in seeds that had been chemically pretreated with retardant hormones. Reduction in TTC stainability has also been detected in chemical pretreated seed samples, but with a relatively higher proportion of TTC-stained seeds than in control seed samples. However, this decline

was greatly reduced in NaDK, ASA, and SADH pretreated seed samples. Regardless of the concentrations used, the extent of the decrease was smaller in all NaDK pretreated seed lots. In comparison to the other samples, the NaDK-induced effect on the maintenance of TTC stainability of all seed forms was observed to be highly important at all observation periods of 0–, 90–, 180–, 270–, and 360–days. A drastic drop compared to chemical pretreated seed samples was recorded in the control samples.

Effect on total dehydrogenase activity (Table 1.5)

Dehydrogenase activity declined steadily in both controlled and chemically pre-treated seed samples with the advance of accelerated ageing days of 0-, 90-, 180-, 270-, and 360-days. In a controlled sample, pretreated seed samples were found to have higher levels of 0-day dehydrogenase activity, and with advances in ageing times, the activity of enzymes declined dramatically, but in chemical pretreated samples, this massive decline was relieved. In all varieties of seed samples, regardless of concentration, the chemical sodium dikegulac appeared to be the most effective, followed by ASA and then SADH. In the case of NaDK (1000 µg/ml), ASA (500 µg/ml) and SADH (300 µg/ml), pretreated seed samples seem more efficient.

Effect on percentage germination (Table 1.6)

The percentage of germination from seed was observed to be higher at around 100% and lower at around 80% after 0 days of pretreatment, but the value gradually decreased as the ageing progressed. The deleterious effects of accelerated ageing were found both in control and pre-treatment samples, but the apparent results of the comparatively higher percentage of germination in pre-treated chemical seed lots which were subjected to accelerated ageing treatment, particularly Na-DK, were mixed. The impact was severe, and the decreasing drift in control samples was found to be more pronounced. In the case of NaDK (1000 µg/ml), ASA (500 µg/ml) and SADH (300 µg/ml), pretreated seed samples seem more efficient.

Effect on protein content (Table 1.7)

In both control and chemically pretreated seed samples in all five varieties, the protein content of seed kernels decreased dramatically during the first, i.e., zero-sampling period. As the days of accelerated ageing progressed in the case of control, the magnitude of loss was continuous. The magnitude of

protein loss was significantly slowed in chemically pretreated seed samples, despite the fact that the chemical impact seemed to be negative at first, as shown by the Na-dikegulac-induced major reduction in protein level. However, the pre-treating chemicals helped to maintain the protein content to a significant degree at subsequent observation times at all concentrations. The chemical initially reduced the protein level over control values during the first 0 days of the sampling period. However, the adverse effects were erased during the second and third sampling periods, i.e., 90-d and 180-d, and the chemical partially adverse effects during the next two sampling periods, i.e., 270-d and 360d, as protein levels in chemical treated samples were higher than in control samples. The chemical sodium dikegulac appeared to be the most effective, followed by SADH and then ASA. The same pattern has also been recorded in sodium dikegulac pretreated seed samples, except that levels were higher compared with other samples after accelerated ageing and seemed to be most effective in that regard in all seed samples, regardless of their concentrations. Pretreated seed samples appear to be more efficient in the cases of NaDK (1000 µg/ml), ASA (500 µg/ml), and SADH (300 µg/ml).

Effect on free amino acid content (Table 1.8)

With the advance of ageing time, free amino acid levels in seeds decreased in control seed samples of all rice seed varieties. The same pattern was observed in chemically pretreated seed samples, but the rate of decrease was significantly slowed at all concentrations. The same pattern was observed in sodium dikegulac pretreated seed samples, except that the amount of free amino acid after 360 days of accelerated ageing was higher than the rest of the samples and appeared to be the most active in this regard in all seed samples, regardless of their concentrations. Here too, NaDK (1000 µg/ml), ASA (500 µg/ml) and SADH (300 µg/ml) pretreated seed samples seem more efficient.

Effect on soluble carbohydrate content (Table 2.9)

In all seed tests, the content of soluble carbohydrates in seed kernels has been increased significantly by 360-days of accelerated ageing. Through the passage of time, the amount of soluble carbohydrate in control seed samples increased gradually up to 180- days, then decreased after 270- and 360- days. Soluble carbohydrate levels in chemical-pretreated seed samples increased to 90-days, then decreased to 180-, 270-, and 360- days. The same pattern has been observed in Na-Dikegulac pretreated seed samples, except that the rate was much slower. Na-dikegulac raised the amount in all seeds over control and effectively retarded the rapid rise of soluble carbohydrates, which seemed to be more promising in this

operation. In this case also, higher concentrations of SADH (300µg/ml) and ASA (500µg/ml) were found more efficient, but in the case of NaDK (1000µg/ml), the lower concentration of chemical was more efficient.

Effect on insoluble carbohydrate content (Table 2.10)

In contrast to changes in soluble carbohydrates, the insoluble carbohydrate content decreased dramatically as seeds were prolonged aged. While carbohydrate levels decreased over control samples in pretreated seed samples, irrespective of their concentrations. The amount in all the seeds analysed following forced ageing remained significant in all the concentrations of Na-dikegulac. Here, NaDK (1000µg/ml), ASA (500µg/ml) and SADH (300µg/ml) pretreated seed samples seem more efficient.

Effect on RNA and DNA content (Table 2.11 and 2.12)

In all seed samples, it was noted that improvements in levels of RNA and DNA had almost the same pattern as protein ones. The data explicitly shows that the amount of RNA and DNA in both the control and chemical pre-treated samples decreased as the days of accelerated ageing progressed. The declining drift in control samples appeared to be substantially increased with prolonged ageing, while in pre-treated samples, regardless of the concentrations used, the declining drift appeared to be significantly slowed. However, this decline was greatly reduced in NaDK, ASA, and SADH pretreated seed samples. The extent of the decrease was smaller in all NaDK pretreated seed lots. In comparison to the other samples, the NaDK-induced effect on the maintenance of RNA and DNA levels of all seed forms was highly maintained at all observation periods of 0-, 90-, 180-, 270-, and 360-days. Here too, NaDK (1000 µg/ml), ASA (500µg/ml) and SADH (300µg/ml) pretreated seed samples seem more efficient.

Effect on catalase enzyme (Table 2.13)

In both control and chemically treated samples, catalase enzyme activity was shown to decrease as the accelerated ageing period progressed. A drastic drop compared to chemical pretreated seed samples was recorded in the control samples. Chemical pre-treated samples appeared to be greatly slowed down in comparison to control samples, despite the decrease in enzyme activity. The same pattern has been observed in Na-Dikegulac pretreated seed

samples, except that the enzyme activity was higher than in other samples. Na-dikegulac seemed to be more promising in this operation. In this case also, higher concentrations of SADH (300µg/ml) and ASA (500µg/ml) were found more efficient, but in the case of NaDK (1000µg/ml), the lower concentration of chemical was more efficient.

Effect on protease enzyme (Table 2.14)

The activity of the catabolic enzyme protease steadily increased both in control and pretreated seed samples with ageing duration. In comparison to the control samples, the chemical-induced amelioration of the increased enzyme activity was more noticeable at all concentrations of NaDK-treated seed samples. Na-dikegulac raised the enzyme in all seeds over control and effectively seemed to be more promising in this operation. Although the improvement of increased enzyme activity was more evident in higher concentrations of samples pretreated with ASA (500µg/ml) and SADH (300µg/ml) chemical samples, in the case of NaDK (1000µg/ml) of the two concentrations used, the lower concentration seems more efficient.

Effect on IAA Oxidase (Table 2.15)

A simple reverse picture has been observed in relation to the general improvements in IAA oxidase function. There was also a substantial increase in IAA production in both control and chemical pretreated seed samples, as observations were made after increased ageing of seed samples. During the first three sample periods, the activity of the enzyme increased, and the enzyme activity dropped drastically during the fourth sampling year. However, during the fifth following sample year, the activity of the enzyme again increased suddenly. Chemical pretreatment samples at various concentrations were shown to be almost equally effective in preventing significant loss of enzyme activity. In all varieties of seed samples, regardless of concentration, the chemical sodium dikegulac appeared to be the most effective, followed by SADH and then ASA. In the case of NaDK (1000 µg/ml), ASA (500 µg/ml) and SADH (300 µg/ml) pretreated seed samples seem more efficient.

Effect on amylase enzyme (Table 2.16)

Amylase activity gradually decreased with the advance of accelerated ageing days in seeds of controlled and pre-treated samples. The declining drift in control samples appeared

to be substantially increased with prolonged ageing. The rate of decrease in activity, on the other hand, was found to be sluggish in seed samples that had been chemically pretreated. Despite such sudden changes in enzyme activity, Na-dikegulac was found to be very effective and to have higher levels of amylase in all cases, and the result was statistically important. Here too, NaDK (1000µg/ml), ASA (500µg/ml) and SADH (300µg/ml) pretreated seed samples seem more efficient.

Effect on superoxide dismutase (Table 2.17)

When it comes to the total dismutase levels, the same image can be seen for the changes in the amylase enzyme. The amount of superoxide dismutase enzymes decreased as the days passed, and the enzyme's activity decreased significantly in both control and chemically pretreated seed samples. However, there has been a steady decline in the activity of dismutase in NaDK, ASA, and SADH pretreated seed samples. The extent of the decrease was smaller in all NaDK pretreated seed lots. In comparison to the other samples, the NaDK-induced effect on the maintenance dismutase level of all seed forms was maintained at all observation periods of 0-, 90-, 180-, 270-, and 360-days. Here too, NaDK (1000µg/ml), ASA (500µg/ml) and SADH (300µg/ml) pretreated seed samples seem more efficient.

PHASE: III

Effect of accelerated ageing after 360 days of seeds of aromatic rice types Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia, pretreated with Ascorbic Acid (ASA, 250 and 500µg/ml)), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml)) and Sodium Dikegulak (NaDK 1000 and 2000µg/ml) on changes in growth and metabolism of plants at five developmental stages [pre flowering (P), flowering (F), seed formation (S), seed mature (M) and pre harvesting (H)] and their impact on crop yield.

Effect on RNA level (Table 3.18)

The data clearly showed that RNA content in leaves increased from the pre-flowering stage (P) to the second sampling time, i.e., during flowering (F), and to the third period of seed forming stage (S) significantly in both control and chemically pretreated seed samples. However, the chemical showed the same pattern as the control during the two following

sampling stages, i.e., seed mature stage (M), which correlates to the pre-harvesting stage (H), and markedly declined. Chemically pre-treated seed samples, on the other hand, partly preserve the levels of RNA in leaves after being exposed to accelerated ageing treatment. This is shown by higher levels of biochemical parameters in plants derived from chemically processed crops. It was discovered that NaDK pretreated plant samples contain more RNA than the other samples.

Effect on DNA level (Table 3.19)

With the progression of plant age, DNA levels in leaves were observed to be reduced in both the control and chemical pretreated leaves of rice plants, which were developed through accelerated ageing treatment. The DNA level decreased remarkably in the case of control plant samples. The rate of fall of pretreated plant samples, on the other hand, was found to be sluggish. Regardless of the decreasing activity of enzymes, it has been determined that Na-dikegulac is more effective than control and other rice plants.

Effect on Soluble Carbohydrate levels (Table 3.20)

The levels of soluble carbohydrate content in the leaves of the five sampling periods decreased first in three subsequent sampling periods, namely pre-flowering (P), flowering (F), and seed formation (S). However, there was a sharp rise in the next two following stages: seed mature stage (M) and pre-harvesting stage (H). Despite the rapid ageing of seed samples, the chemical has been shown to partly maintain the levels of carbohydrate throughout the leaves. The findings of the Na-dikegulac pretreated plant samples were observed to be better than the control plant samples.

Effect on Insoluble Carbohydrate levels (Table 3.21)

The data on the insoluble carbohydrate level, in contrast to the soluble carbohydrate content, showed a negative outcome. During the pre-flowering (P), flowering (F), seed development (S), and seed mature stage (M) sampling stages, there was a steady increase in levels, but during the pre-harvesting stage (H), there was a reduction in the levels of plants developed from accelerated aged seed samples. The reduction of activities was partly halted in plants developed by chemically pretreated seeds. The pretreatment with Na-dikegulac was found to be more effective than the control.

Effect on Catalase levels (Table 3.22)

The activity of the catalase enzyme decreased as the sampling period progressed in both control and chemical pretreated samples. The same pattern has been observed in ASA, SADH, and NaDK pretreated plant samples, except that the enzyme activity was higher than control samples. Here also, the enzyme level in Na-dikegulac pretreated samples was observed to partly arrest the loss of enzyme activities compared to the control and other samples and seemed to be more promising in this operation.

Effect on activities of Protease enzyme (Table 3.23)

The activity of the catabolic enzyme protease followed a nearly similar pattern to that of catalase. Protease levels in both control and chemically pretreated samples decreased significantly as the sampling cycle progressed, but there was a sudden increase in enzyme levels in chemically pretreated samples during the final sampling period. The amount of enzymes found in Na-dikegulac is higher than in other plant samples and appears to be more effective.

Effect on the activities of IAA-oxidase enzyme (Table 3.24)

Over five sampling periods, IAA-oxidase enzyme levels decrease in leaves of plants grown from accelerated ageing, both in control and chemically pretreated samples.The reduced level of this enzyme has been effectively retarded by the pre-treatment chemicals. Regardless of the decrease in enzyme activity, the enzyme level in chemically pretreated samples was found to be higher and more effective than control samples. The amount of enzymes found in Na-dikegulac is higher than in other plant samples and appears to be more efficient.

Effect on the activities of RNAse enzyme (Table 3.25)

The activity of RNAse in both pre-treated chemicals and in control samples showed a continuous increase with the progression of three corresponding sample periods (P), (F), and (S). However, the next following sampling point, (M), revealed a decline in enzyme activity, followed by a sudden rise in the (H) stage in the final sampling period. Regardless of the different results in enzyme activity, chemical pretreated plant samples were found to be more effective than the control samples. Na-dikegulac raised the amount of all seeds over control effectively and seemed more promising in this operation.

Effect on the activities of superoxide dismutase enzyme (Table 3.26)

The activities of superoxide dismutase enzymes were reduced significantly and proportionally in the leaves of plants grown from accelerated aged seed samples. But for the (M) sampling period, there was a sudden increase in activity across all five sampling periods. Regardless of enzyme activity, the enzyme level in Na-dikegulac pretreated samples was observed to be higher than in the other remaining samples.

Effect on yield attributes (Table 3.27)

Data indicated that when plants were grown from accelerated aged seeds, yield attributes such as overall yield per panicle and 1000 seed weight were improved significantly compared to control, resulting in a significant improvement in crop yield. In contrast to control samples, seed pretreatment with Na-dikegulac resulted in a significant improvement in crop yield.

Effect on plant height (Table 3.28)

Results revealed that in germination, the impact of chemical pretreatment and accelerated ageing has been significantly retarded and germinated in all seed samples over 7-9 days. In the case of control, the plants' heights were remarkably reduced before the shift of plants when the plants were raised from accelerated aged seeds. However, the chemical showed the same pattern but its impact was not the same as that of control. When the plant was shifted, the height was significantly induced in chemically pretreated plants, regardless of concentration, and we checked height reduction. In contrast to the control plants, Na-dikegulac performs better for all five developmental stages.

Effect on stem circumference and inter-nodal elongation (Table 3.29)

Inter-nodal elongation was significantly induced after plant shifting in all chemically pretreated plants. The reduction in the internodal elongation compared with the control plants has been checked with chemical pretreated plants irrespective of their concentrations. Concurrently, the pretreated chemical inhibited the ageing-induced retardation of stem diameter, and this influence of Na-dikegulac was shown to be consistent throughout all five developmental phases.

Table 2 :Analysis of rice grain quality of 14 different aromatic rice seed varieties.

Varieties of aromatic rice seeds	Size Scale	Shape Scale	Grain Appearance	Degree of elongation	Aroma	Alkali digestion	Gelatinization Temperature	Score	Amylose content
1.Kalo Nunia	3	1	0	1.3	Strong	Low	High	1	11.74
2.Addey	5	5	0	1	No	Low	High	2	13.49
3.Mansari	3	5	0	1.2	Moderate	Low	High	1	7.9
4.Kattaka	3	5	0	1	Slight	Intermediate	Intermediate	5	14.98
5.Raj Bahara	3	5	0	1.1	Slight	Low	High	1	14.46
6.Mohan Bhog	3	5	0	1.5	Strong	Intermediate	Intermediate	4	13.38
7.Khemti	1	5	1	1.2	Moderate	High	Low	7	11.51
8.MasinoBasmati	3	5	1	1.2	Strong	Low	High	1	11.44
9.Krishna Bhog	5	9	0	1.3	Moderate	Low	High	2	11.37
10.Musli	3	1	0	1.6	Slight	High	Low	6	14.09
11.Dudhraj	3	5	1	1.1	Moderate	Intermediate	High	3	10.02
12.Fulpaty	5	9	0	1.1	Moderate	Intermediate	Intermediate	4	9.32
13.Arya	1	5	0	1.6	Slight	Low	High	1	15.91
14.Birimful	3	5	0	1.2	Moderate	Low	High	1	18

Table 3 : Growth phases of aromatic rice plant life cycle. Data were recorded from five uniformly grown plants.

Growth phases of rice plant		Days required after sowing	Remarks
1. Vegetative Growth			
Stage 0	Germination to emergence	13±3 days	Germination begins with the appearance of the young shoot and root through the seed coat at one end of the seed. By the second week after seedling in the seedbed, the first leaf breaks through the coleoptile.
Stage 1	Seedling	24 ± 9 days	During this stage, seminal roots and up to five leaves develop. Seedling stages occur during the first two to three weeks after planting and ready for transplanting.
Stage 2	Tillering	65 ± 25 days	This stage extends from the appearance of the first tiller until the maximum tiller number is reached. Tillers emerge from the axiliary buds of the nodes and displace the leaf as they grow and develop. This occurs after transplanting. The plant is now increasing in length and tillering very actively.
Stage 3	Stem elongation	75 ± 65 days	Growth duration is significantly related to stem elongation. In this respect, rice varieties can be categorized into two groups: the short-duration varieties which mature in 105-120 days and the long-duration varieties which mature in 150 days.
2. Reproductive growth			
Stage 4	Panicle initiation to booting	77 ± 4 days	The initiation of the panicle primordium at the tip of the growing shoot becomes visible to the naked eye. As the panicle continues to develop, the spikelets become distinguishable. The young panicle upward extension inside the flag leaf sheath causes to bulge, called booting. Booting is most likely to occur first in the main culm.
Stage 5	Flowering	88 ± 7 days	At flowering, the florets open, the anthers protrude from the flower glumes because of stamen elongation, and the pollen is shed. It takes about 7 days for all spikelets in a panicle to open.
3. Grain ripening			
Stage 6	Milk grain stage	100 ± 5 days	The grain starts to fill with a white, milky liquid, which can be squeezed out by pressing the grain between the fingers. The panicle looks green and starts to bend.
Stage 7	Dough grain stage	125 ± 15 days	The milky portion of the grain first turns into soft dough and later into hard dough. The grains in the panicle begin to change from green to yellow. The field starts to look yellowish as the panicle turns yellow.

PHASE I

Table 1.4: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on percentage TTC- stained in seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seed lots were kept in 95% RH. Generalized stain-abilities were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	98	44.6	28.8	27.2	18.2	99.3	50.4	51.5	20.4	14.3	63.6	39.6	20.2	12.2	12.8	94.6	45.3	32	20	12	52.3	30.2	19.7	10.9	10.1
ASA (250)	98.6	94.3	55.3	44	29.3	86.3	86.3	65.1	52.3	27.3	73	53.6	47.3	29.3	22	100	90.3	60.3	49.7	20.7	55	34.5	33	22	17.1
ASA (500)	95.3	94.6	57.3	45.6	39.2	90.3	76.6	61.6	39.3	22.2	98.6	66.7	50.3	47.2	26.6	95	73.2	52.3	37.6	26.2	53.6	33.3	29.6	20.4	18.3
SADH (150)	95.3	77.6	65.6	61.6	39.5	94.3	73.3	64.6	55.3	22.6	94.3	65.6	51.6	44.4	33.6	94.3	65.5	57.3	47.3	33.3	43.6	34.3	27.6	23.6	17.6
SADH (300)	95.3	88.3	61.8	54.3	44.6	95.6	65.6	55.4	50.4	26.8	94.6	76.6	64.6	50.6	47.3	93.3	76.6	68.5	54.6	34.6	40.2	32.1	27.6	22.1	22.5
NaDK (1000)	98.3	94.6	73.2	59	57.3	95.3	79.1	65.6	61.6	37.6	98.6	90.3	75.5	65.6	55.3	98.6	94.3	73.3	65.6	54.5	64.6	51.2	44.6	37.1	34.2
NaDK (2000)	95.6	79.9	66.1	57	47.3	98.6	73	68.2	57.3	33.2	95.3	86.3	61.4	55.3	51.1	99.3	95.3	77.6	62.3	45.5	65.2	50.1	41.3	35.1	32.1
LSD (P=0.05)	4.765	2.23	1.44	1.36	0.91	4.315	2.52	2.575	1.02	0.715	3.18	1.98	1.01	0.61	0.64	4.665	2.265	1.6	1	0.6	2.01	1.51	0.985	0.545	0.505

Table 1.5: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on dehydrogenase content in seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked and treated with chemicals for 6 hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	697	545	218	175	107	593	511	379	233	116	767	548	492	335	212	521	492	318	236	164	517	399	230	184	160
ASA (250)	780	559	546	380	335	546	438	425	374	268	469	462	442	307	221	523	502	390	274	175	424	353	317	293	193
ASA (500)	652	489	452	387	355	892	581	482	379	276	502	443	438	412	226	518	468	381	276	265	612	602	317	218	129
SADH (150)	398	375	390	311	218	592	502	375	238	210	506	502	405	380	284	482	470	455	412	272	431	307	289	218	176
SADH (300)	542	498	482	394	224	481	451	434	391	268	482	434	474	412	391	482	442	378	367	364	334	269	211	200	193
NaDK (1000)	647	646	608	517	456	702	577	571	474	422	593	571	506	457	442	780	780	652	518	442	783	545	358	311	265
NaDK (2000)	811	619	545	523	454	622	572	546	444	384	680	580	558	506	468	942	782	694	515	410	677	373	339	311	252
LSD (P=0.05)	19.9	18.75	19.5	8.75	5.35	24.05	21.9	18.95	11.65	5.8	23.45	21.7	20.25	15.35	10.6	24.1	22.1	15.9	11.8	8.2	16.7	13.45	11.5	9.2	6.45

Table 1.6: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on percentage germination of seeds

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seed lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	99.3	76.6	55.7	20.4	11.6	97.6	70.7	48.9	22.6	11.6	100	69.6	49.7	24.6	13	98	78	44.7	26.3	16.66	79.1	59.31	36.1	22.1	18.1
ASA (250)	100	89.6	68.6	31.6	23.6	100	89	68.3	33.3	23.33	94.5	86.6	69.4	31.8	16.66	100	84.7	70.6	41	21.6	82.1	67.2	53.1	36.1	25.1
ASA (500)	100	88	62.8	35.4	26.6	99.6	76.5	54.2	38.8	27.5	100	78.3	58.6	34.4	26.6	98.6	88.6	68.2	33.6	23.6	83.4	68.3	54.1	35.1	26.9
SADH (150)	95.6	81.3	66.8	33.6	22.6	99.3	75.4	51.8	34.3	22.6	100	80.2	64.4	34.5	23.4	100	88.2	52.8	33.6	26.66	79.1	67.5	51.1	33.1	24.4
SADH (300)	100	82.6	61.8	38.3	24.7	100	82.6	63.4	38.2	26.66	100	86.6	66.7	35	22.2	100	86.3	61.3	32.5	22.6	80.1	68.2	57.1	34.1	25.1
NaDK (1000)	100	91.6	76.4	59.3	37.3	100	93	70.6	52.6	36.9	100	90.3	71.6	52.1	34.3	99.9	81.33	67.6	47.6	35.6	88.4	81.2	68.1	44.1	32.1
NaDK (2000)	100	88.3	68.8	52.6	34	100	90.3	67.3	44.6	33.2	99.3	83.6	64.7	47.3	31.6	100	86.4	64.8	43.6	33.3	90.8	81.5	69.1	45.6	30.1
LSD (P=0.05)	4.78	3.83	2.785	1.02	0.58	4.88	3.535	2.445	1.13	0.58	4.725	3.48	2.485	1.23	0.65	4.9	3.9	2.235	1.315	0.833	3.955	2.9655	1.805	1.105	0.905

Table 1.7: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on protein content of seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	190	184	164	144	135	199	179	166	159	139	198	180	160	143	127	195	188	166	156	122	191	178	164	152	139
ASA (250)	181	161	281	203	149	153	192	230	213	160	141	172	282	236	150	159	195	283	232	191	129	156	291	225	149
ASA (500)	138	190	277	264	187	154	188	247	202	189	159	173	266	244	192	196	209	319	280	245	153	197	262	246	152
SADH (150)	147	129	149	242	165	190	271	313	230	197	189	200	236	205	155	191	267	304	292	215	109	147	249	194	164
SADH (300)	140	161	260	192	152	195	220	289	241	205	154	214	344	241	157	136	163	295	221	152	118	164	231	189	167
NaDK (1000)	163	200	265	226	210	151	178	281	251	206	125	162	225	205	197	167	193	262	228	200	191	149	249	215	180
NaDK (2000)	178	206	235	215	200	179	197	273	247	208	137	167	173	200	152	158	189	289	248	193	148	162	250	233	176
LSD (P=0.05)	6.9	6.45	7.45	7.2	6.75	7.55	8.9	8.3	7.95	6.95	6.25	8.1	8	7.15	6.35	6.8	8.15	8.3	7.8	6.1	5.45	7.35	8.2	7.6	6.95

Table 1.8: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on free amino acid content of seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	67.8	58.8	35.5	20.7	11.7	57.5	39.6	22.2	17.01	7.42	64	50.4	38.57	25.93	11.4	44.4	36.4	25.7	18.7	10.7	42.08	31.7	29.82	17.7	9.67
ASA (250)	129	64.71	37.02	25.56	15.56	55.8	43.6	37	26.45	17.3	115	80.4	63.5	30.4	17.2	109	89.42	61.6	26.5	13.31	103	98.5	64.4	24.9	12.9
ASA (500)	126	67.2	44.2	26.21	16.21	71.8	53.2	31.8	20.7	11.8	150	125	83.6	31	12.1	91.4	61.4	48.6	24.7	12.6	129	113	87.9	37.02	19.8
SADH (150)	78.6	53.8	31.8	27.57	17.57	87.3	60.7	48.6	28.5	14.91	92.3	62.3	49.7	27.54	19.7	88.9	64.9	39.31	24.9	12.41	95.5	60.2	38.8	29.2	15.25
SADH (300)	82.5	72.6	49.94	29.71	19.71	82.6	68.4	54.4	31.6	18.57	65	21.8	18.4	21.8	12.6	99.4	74.5	58.68	28.9	14.5	118	75.9	40.7	28.2	15
NaDK (1000)	92	78.6	52.6	33.1	29.2	94.8	63.3	48.4	38.4	27.48	73.8	53.1	44.7	34.4	24.7	64.3	64.3	44.1	37.4	23.8	98	81.4	66	10.5	26.1
NaDK (2000)	86	67.2	46	31.1	26.4	91.07	75.8	42.6	38.11	25.45	87.9	62.8	50.6	37.8	28.8	88.9	50.6	49.4	33.4	20.6	96	84.6	71	37.5	24.15
LSD (P=0.05)	3.39	2.69	1.59	1.035	0.585	2.79	1.98	1.11	0.8505	0.371	3.2	1.09	0.92	1.09	0.57	2.22	1.82	1.285	0.935	0.535	2.104	1.585	1.491	0.885	0.4835

PHASE II

Table 2.9: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on soluble carbohydrate content of seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	0.34	0.44	0.5	0.32	0.12	0.31	0.41	0.35	0.24	0.13	0.22	0.29	0.42	0.15	0.11	0.31	0.35	0.4	0.2	0.13	0.32	0.44	0.56	0.39	0.14
ASA (250)	0.29	0.36	0.28	0.23	0.15	0.38	0.47	0.37	0.29	0.15	0.3	0.53	0.39	0.24	0.12	0.45	0.66	0.52	0.39	0.18	0.11	0.59	0.23	0.6	0.25
ASA (500)	0.26	0.47	0.33	0.21	0.19	0.35	0.39	0.31	0.26	0.18	0.26	0.54	0.45	0.32	0.13	0.37	0.42	0.38	0.28	0.17	0.24	0.4	0.32	0.29	0.21
SADH (150)	0.44	0.63	0.39	0.21	0.14	0.46	0.59	0.45	0.38	0.27	0.6	0.2	0.13	0.08	0.32	0.27	0.35	0.22	0.06	0.11	0.26	0.43	0.16	0.26	0.31
SADH (300)	0.42	0.52	0.39	0.23	0.17	0.37	0.48	0.35	0.27	0.17	0.36	0.43	0.22	0.4	0.21	0.51	0.67	0.52	0.39	0.22	0.37	0.2	0.3	0.13	0.3
NaDK (1000)	0.47	0.72	0.51	0.38	0.19	0.43	0.76	0.59	0.39	0.26	0.54	0.74	0.57	0.35	0.17	0.53	0.63	0.56	0.37	0.21	0.53	0.67	0.51	0.38	0.18
NaDK (2000)	0.4	0.65	0.42	0.28	0.16	0.52	0.82	0.65	0.35	0.24	0.65	0.76	0.57	0.41	0.18	0.89	0.68	0.61	0.48	0.22	0.59	0.73	0.47	0.34	0.23
LSD (P=0.05)	0.013	0.018	0.014	0.010	0.006	0.015	0.019	0.015	0.012	0.006	0.011	0.014	0.006	0.004	0.005	0.013	0.017	0.011	0.003	0.005	0.005	0.01	0.008	0.006	0.007

Table 2.10: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on Insoluble carbohydrate content in seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	17.7	15.1	13.5	12.8	10.9	18.6	14.3	11.5	10.1	3.78	16.5	14.5	13.5	13.3	9.64	19.2	14.2	13.9	12.3	8.94	17.8	14.4	13.3	12.4	4.94
ASA (250)	14.6	13.8	13.6	12.6	12.4	14.9	13.4	13.3	12.9	12.2	14.3	12.9	11.3	11.1	10.7	15.1	13.6	12.4	12.1	11.6	16.6	14.1	13.1	12.8	12.2
ASA (500)	15.6	14.7	12.4	12.2	10.2	15.4	14.8	14.6	13.5	12.8	14.4	14	13.9	13.9	11.7	14.9	14.6	13.9	12.2	12.1	16.3	16	14.4	13.8	12.7
SADH (150)	17.5	15.4	12.7	12.6	12.5	17	14.1	12.8	11.8	11.6	13.8	15.5	14.3	11.5	11.4	19	16.3	15.2	14.4	13.3	17.4	14.5	12.7	11.4	11.3
SADH (300)	17.4	16.3	14.6	11.8	11.3	18.3	18	13.5	12.2	11.3	14.3	16	13.2	12.4	12	17.7	17	13.8	12.6	12.3	16.7	17.4	14.8	12.4	12.3
NaDK (1000)	15.7	15.1	14.6	14.2	13.9	18	17.2	15.4	14.9	13.2	16	15.8	15.4	14.9	14.1	18.7	16.6	15.2	15	14.9	16.9	15.4	14.8	14.1	14
NaDK (2000)	16.3	14.9	14.5	12.3	12.9	16.2	15.1	14.3	13.6	12.6	14.5	14.8	14.1	13.2	12.6	17	16.7	14.4	14	13.6	15.7	16.5	16.4	14.4	13.1
LSD (P=0.05)	0.73	0.69	0.62	0.59	0.545	0.745	0.67	0.575	0.505	0.189	0.69	0.645	0.565	0.555	0.482	0.745	0.68	0.62	0.605	0.447	0.785	0.705	0.635	0.57	0.247

Table 2.11: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on RNA content in seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	42.6	37.4	27.4	17.2	3.18	43.8	38.5	25	14.7	2.97	43.8	22.4	19.7	10.7	3.34	41.7	28.8	21.6	15.8	9.6	41.5	37.3	23.3	19.5	7.42
ASA (250)	39.3	21.4	18.2	11.5	7.47	47.1	31.4	20.5	17.9	5.53	41.3	30.6	20.9	15.6	6.5	44	39.16	26.5	18.1	11.6	42	30.7	28.9	16.7	7.47
ASA (500)	47.2	38.8	22	18.1	6.75	49.5	38.9	29.2	22	9.04	45.3	37.7	28.1	19.7	6.8	47.9	41.3	32.4	21.8	12.2	37.6	30.2	27.2	17.6	11.1
SADH (150)	37.1	23.3	20.9	11.4	5.9	30.5	21.3	19.4	10.5	6.02	40.1	30.4	21.1	10.6	7.1	36.1	21.6	25.3	16.2	10.2	34.3	29.5	26.2	20.9	11
SADH (300)	38.9	28	21.1	12.9	6.23	39.8	29.5	20.7	19.6	7.88	32.7	21.7	18.4	16.3	8.72	39.6	31.6	20.9	18.4	11.7	35.5	28.4	21	18.2	10.3
NaDK (1000)	42.6	35.9	25.1	23.6	14.4	40.5	30.2	26.7	24.2	14.2	40.3	29.5	21.1	18.3	13.9	35.1	29.4	25.3	20.04	15	41.2	34.99	26.8	20.1	14.6
NaDK (2000)	44.1	31	21.9	18.1	12.1	38.3	34.6	26.1	21.1	13.8	32.5	28.2	21.2	16.2	14.5	35.7	27.7	22.6	15.7	12	43.8	36.5	29.5	16.2	12.2
LSD (P=0.05)	1.855	1.07	0.91	0.57	0.295	1.525	1.065	0.97	0.525	0.1485	1.625	1.085	0.92	0.53	0.167	1.755	1.08	1.045	0.785	0.48	1.715	1.42	1.05	0.81	0.371

Table 2.12: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on DNA content in seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	11.3	7.2	4.2	3.19	1.27	14.2	9.86	7.97	3.84	1.88	8.55	7.39	4.13	3.33	1.04	10.8	6.38	4.27	2.75	1.88	8.7	6.81	4.78	1.95	0.92
ASA (250)	8.7	7.83	5.87	3.19	I.9	8.99	8.41	3.98	2.9	2.01	8.84	7.39	4.27	3.48	3.04	8.41	7.1	4.64	2.74	2	8.55	6.96	3.11	2.39	1.15
ASA (500)	9.13	8.7	3.3	2.97	2	8.41	7.83	3.26	3.04	2.17	8.84	8.41	2.97	2.32	1.88	10.2	8.12	5.07	3.75	2.9	8.77	8.41	4.64	3.7	2.45
SADH (150)	9.3	7.1	5.28	3.01	1.59	11.8	9.57	8.55	8.12	3.74	11.2	8.12	4.78	3.59	2.17	11.09	9.57	6.58	5.8	2.32	11.4	7.68	4.54	3.43	2.24
SADH (300)	11.2	9.41	7.83	5.5	2.62	14.8	10.2	8.12	4.2	2.17	13	11.5	7.54	4.64	3.48	12	11.3	8.7	5.25	2.58	11	9.05	5.28	4.43	2.58
NaDK (1000)	10.8	8.88	6.33	5.96	5.58	10.13	9.7	5.29	4.17	3.39	13.8	9.42	7.68	6.54	5.04	13.6	10.1	7.83	5.97	4.13	10.5	7.54	5.25	4.62	3.11
NaDK (2000)	10.7	8.41	6.88	5.84	4.06	11.6	7.25	4.27	3.18	3.11	13.5	10.1	7.97	5.91	4.28	12.3	10	8.84	4.84	3.13	13.9	9.28	7.42	4.28	3
LSD (P=0.05)	0.435	0.36	0.165	0.148	0.063	0.42	0.362	0.163	0.145	0.094	0.427	0.369	0.1485	0.116	0.052	0.42	0.319	0.213	0.137	0.094	0.427	0.34	0.155	0.097	0.046

Table 2.13: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on catalase content in seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	287	62.4	56.7	21.9	6.6	155	63.9	58.2	21	9.6	153	57.3	57.9	22.2	11.7	172	70.8	58.2	17.7	8.4	118	63.4	55.8	21.6	12.6
ASA (250)	363	234	42.3	38.4	18.6	372	279	150.4	45.6	22.9	359	246	166	51.8	25.2	369	299	165	54.7	27.3	357	241	160	60.6	19.8
ASA (500)	371	240	114	36	33.3	360	263	110	41.4	24	370	212	108	63.7	26.7	370	216	146	67.3	28.5	369	216	135	61.9	23.7
SADH (150)	376	233	56.4	28.8	9.9	371	215	115	36	23.1	363	292	110	53	24.3	306	248	115	71.8	21.8	364	232	133	58.5	26.1
SADH (300)	348	238	43.2	36.6	18.4	354	297	129	69.6	29.3	342	236	178	62	30.6	359	279	143	62.1	22.1	358	252	139	68.7	29.4
NaDK (1000)	343	290	166.3	69.2	32.7	262	104	81.7	69.4	34.6	326	232	163	78.9	32.6	371	293	157	69.9	31.9	277	170	92.1	72.2	32.4
NaDK (2000)	243	90.3	86.4	65.7	30.9	286	188	88.8	66.5	30.9	256	121	79.2	45.5	29.7	254	121	80.7	60.6	29.3	216	162	86.5	52.4	31.8
LSD (P=0.05)	12.15	3.12	2.115	1.095	0.33	7.75	3.195	2.91	1.05	0.48	7.65	2.865	2.895	1.11	0.585	8.6	3.54	2.91	0.885	0.42	5.9	3.17	2.79	1.08	0.63

Table 2.14: Effect of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on Protease content in seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	35.9	59.2	75.3	90.6	105	26.4	61	72.9	94.7	102	26.8	52.5	63.4	76.5	99.9	23	52.2	61.4	72.3	88.7	27.8	57.3	75.9	102	112
ASA (250)	41.1	69	71.4	92.4	138	33.6	79.8	93.6	102	130	45	66.3	88.2	106	125	46.2	69.2	83.1	113	126	35.6	72.9	98.3	102	116
ASA (500)	47.7	77.1	89.7	124	145	40.6	74.4	90.3	111	136	55	71.8	91.5	116	127	50	74.1	90	114	127	31.2	67.5	80.4	116	126
SADH (150)	46.2	35.8	68.3	91.5	136	48.9	64.8	93.2	110	134	41.4	67.3	81	104	124	48	63	88	116	131	40.8	68	90.8	107	124
SADH (300)	47.1	36.1	72.9	105	147	45.4	70.2	93	118	137	42.6	74.5	93.7	118	129	41.1	62.9	82.5	118	135	45.2	81.5	101	123	144
NaDK (1000)	41.2	68.1	90.4	129	160	74.4	96.1	106	127	144	50.4	72.9	99.9	124	148	56.3	82.7	103	126	161	48	85.2	106	130	150
NaDK (2000)	49.2	65.5	81.6	117	155	69.6	134	114	124	141	67.5	74.4	102	120	144	53.5	84	93.2	123	153	49.8	88.3	107	125	145
LSD (P=0.05)	1.795	1.79	3.415	4.575	5.25	1.32	3.05	3.645	4.735	5.1	1.34	2.625	3.17	3.825	4.995	1.15	2.61	3.07	3.615	4.435	1.39	2.865	3.795	5.1	5.6

Table 2.15: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on IAA Oxidase content in seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	3.4	5.56	2.96	3.26	3.68	1.58	5.31	2.72	2.1	3.3	0.13	2	2.54	1.64	4.22	1.4	4.86	2.54	2.76	3.36	0.47	2.59	3.32	3.24	3.48
ASA (250)	5.82	4.16	7.8	2.48	4.96	2.15	3.36	7.2	1.82	6.72	4.42	4.07	7.02	1.82	5.46	0.21	5.15	7.52	2.04	5.36	6.62	4.54	5.24	1.86	5.28
ASA (500)	2.23	5.52	6.16	2.68	5.4	0.3	4.35	6.28	2.34	5.18	0.78	5.08	4.16	2.34	5.28	5.82	3.39	7.44	4.02	5.26	6.05	3.75	7.3	1.88	5.28
SADH (150)	1.39	2.02	4.4	2.06	7.08	0.95	2.83	3.5	2.1	7.32	3.87	3.07	4.04	1.84	6.24	0.26	2.2	3.8	1.82	9.26	2.14	3.1	3.92	2.4	7.08
SADH (300)	6.55	2.92	3.1	2.28	10	4.04	4.41	3	2.42	6.94	5.14	1.88	9.82	2.8	7.1	3.76	4.19	3.76	2.32	6.9	3.67	3.89	4.04	2.1	6.54
NaDK (1000)	3.37	3.65	10.1	2.1	7.12	5.59	5.33	10	2.48	8.26	12.5	3.24	9.78	2.7	9.96	7.63	3.5	11.3	2.78	9.28	2.41	2.76	9.46	2.52	9.92
NaDK (2000)	2.47	5.73	11.5	2.4	9.08	5.02	4.91	8.56	3.2	10.6	1.04	5.84	9.82	3.18	10.4	0.34	3.39	8.64	2.84	7.2	0.22	4.19	10.7	2.36	8.98
LSD (P=0.05)	0.0695	0.101	0.148	0.103	0.184	0.015	0.1415	0.136	0.091	0.165	0.0065	0.1535	0.127	0.082	0.211	0.0105	0.11	0.127	0.091	0.168	0.011	0.1295	0.166	0.093	0.174

Table 2.16: Effects of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on Alpha Amylase content in seeds.

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG					KHEMTI					MASINO BASMATI					MUSLI					KALONUNIA				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360	0	90	180	270	360
Control (H_2O)	17.4	8.04	6.68	4.23	0.66	17.6	7.98	6.48	3.78	1.8	15.8	9.24	7.38	3.27	0.72	16.2	10.4	6.72	4.96	1.56	14.6	11.6	6.4	3.06	1.65
ASA (250)	18.1	10.3	9.66	4.12	1.98	18.5	13.8	12.4	12.1	6.05	12.1	10.3	8.76	7.74	6.06	13.6	10.5	9.12	7.26	6.31	17.4	15.2	12.7	8.14	6.88
ASA (500)	12.6	11.8	8.22	7.98	2.13	19.1	15.5	12.9	11.1	5.11	13.2	12	10.6	8.54	6	13.9	11.3	10	8.1	6.17	16.4	14.3	10.3	8.4	5.02
SADH (150)	17.5	13.8	10.8	5.52	3.42	19.3	15.4	13.9	12.7	6.18	11.3	9	7.92	6.6	5.9	14.7	12.7	11.7	9.48	6.02	17.1	15.9	10.1	9.02	6.54
SADH (300)	17	12.5	10.6	4.56	3.6	16.6	14.8	13.8	12.9	7.08	16.5	13.6	10.9	9.7	6.48	15.9	13.4	11.1	8.78	6.51	16.2	15.5	12.5	9.78	6.74
NaDK (1000)	19.9	17.4	13.3	10	16.8	19.2	16.9	14.8	12.9	10.3	16.8	12.7	9.92	8.94	8.58	16.8	14.8	12.8	10.8	9.3	19.2	16.6	14.4	10.2	8
NaDK (2000)	17.4	14.3	11.7	9.78	19.2	18.1	15.9	13.2	11.6	8.58	15.2	11.7	9.92	8.56	7.56	14.6	12.3	11.7	9.84	7.8	17.5	15.7	12.7	11.5	7.2
LSD (P=0.05)	0.63	0.402	0.334	0.206	0.033	0.83	0.69	0.324	0.189	0.09	0.565	0.462	0.369	0.1635	0.036	0.68	0.52	0.336	0.248	0.078	0.73	0.58	0.32	0.153	0.0825

Table 2.17: Effect of pretreated Aromatic rice seeds with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) on Superoxide dismutase content in seeds

A healthy and viable 5 varieties of seeds **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were presoaked with treated chemicals for 6hr and sundried. The treatment of soaking and drying processes were repeated three consecutive times and seeds lots were kept in 95% RH. Generalized data were recorded at 0-, 90-, 180-, 270-, and 360- days after pretreatment.

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	M 0	M 90	M 180	M 270	M 360	H 0	H 90	H 180	H 270	H 360	B 0	B 90	B 180	B 270	B 360	S 0	S 90	S 180	S 270	S 360	K 0	K 90	K 180	K 270	K 360
Control (H_2O)	29.7	27.3	20.5	14.7	2.19	30.9	26.8	14.2	8.49	4.86	24.7	19.4	12.7	7.08	1.17	21.1	13.6	7.59	3.61	1.45	30.7	37.5	1.2	3.36	4.68
ASA (250)	52.6	45.7	30.9	16.9	6.06	36.9	20.9	15.6	11.5	5.46	42.9	39.1	23.2	16.6	5.64	44.3	33.8	21.2	13.1	6.29	42.8	61.8	0.72	7.29	6.45
ASA (500)	50.2	49.4	31.8	18.9	6.24	32.4	32.4	14.3	9.27	6.45	34.5	21.4	17.8	10.6	6.42	41.4	34.3	23.2	12.4	6.27	21.2	41.2	0.9	13.6	6.96
SADH (150)	50.4	45.4	24.05	19.1	6.6	42.6	39.3	27.8	15	7.59	58.8	48.7	36.1	17.4	7.92	39.5	23.4	14.92	9.64	6.72	50	55.4	2.94	7.71	6.9
SADH (300)	45.6	32.5	26.9	17.9	6.57	47.8	34.8	28.3	18.7	8.26	51.9	39.3	26.6	18.1	7.75	36.6	22.4	16.45	8.25	6.84	49.2	54.3	3.87	6.72	6.24
NaDK (1000)	58.1	46.5	34.8	19.9	9.08	54.7	42.6	36.4	26.7	8.87	58	40.5	35.4	19.4	8.02	54.4	42.4	26.6	15.6	7.69	62.9	64.9	4.86	13.2	7.02
NaDK (2000)	64.5	49.9	29.2	16.7	8.93	53.9	49.6	33.1	24.8	7.63	68.9	49.9	34.8	17.5	7.98	59.2	48.8	24.8	16.6	7.17	60.2	63.9	9	3.54	7.41
LSD (P=0.05)	1.485	1.365	1.025	0.735	0.1095	1.545	1.045	0.71	0.4245	0.243	1.235	0.97	0.635	0.354	0.0585	1.055	0.68	0.3795	0.1805	0.0725	1.535	1.875	0.036	0.168	0.234

PHASE III

Table 3.18: Effect of pretreated Aromatic rice with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes RNA (µg/g fresh weight) contents in leaves of rice plant.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at five developmental stages i.e. Pre flowering stage (P), Flowering stage (F), Seed formation stage (S) and seed mature stage (M) and pre harvesting stage (H) which corresponds to 67d, 86d, 105d, 120d and 132d of plant age respectively

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H
Control (H_2O)	30.5	32.4	56.2	19.5	12.3	29.9	45.1	71.3	11.5	10.7	30.5	31.6	34	18.6	13.5	30.1	31.4	44	19.3	12.6	32.9	40.8	56	30.4	19
ASA (250)	36.7	42	52.1	22.3	19.4	26.7	36	44.4	24.4	18.2	25.2	32.5	42.1	25.3	18.4	21.2	31.2	42.2	26.9	17.5	29.9	38.7	43.1	27.9	20
ASA (500)	41.3	50.2	57.5	29.4	20.9	31.7	41.6	72.6	31.6	28.5	32.6	36.2	41.8	28.5	20.6	29	31	44.1	29.2	20.6	30.7	35.3	56.3	30.3	27.2
SADH (150)	37.3	42.9	54.2	22.3	19.7	26.8	35.5	42.7	24.5	15.5	35.9	41.1	45.7	28.2	18.4	25.6	32.4	41.6	27.1	22.5	30.3	42	52.8	39.7	26.7
SADH (300)	36	43.3	57.7	29.1	21	32.1	39	72.3	41.6	28	39.3	43.3	46.9	36.9	23.7	27.8	31.4	44.7	32.6	24.2	32.8	43.3	56.1	30.6	21.7
NaDK (1000)	36.1	42.9	52.3	32.4	28.5	25.8	35.4	49.1	24.6	28.3	37.5	45.7	53	38.2	25.4	27.4	32.4	43.9	37.1	27.6	30.6	40.7	53.2	38	29.8
NaDK (2000)	46.7	52	58.2	29.6	25	32	40	50.5	31.5	28.7	39.7	42.9	50.4	38.6	26.1	28.7	31.1	40.5	32.7	30.1	33.3	41	50.4	30.4	27.3
LSD (P=0.05)	1.525	1.62	2.605	0.975	0.615	1.29	1.77	2.135	0.575	0.535	1.26	1.58	1.7	0.93	0.675	1.06	1.55	2.025	0.965	0.63	1.495	1.765	2.155	1.395	0.95

Table 3.19: Effect of pretreated Aromatic rice with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes DNA (µg/g fresh weight) contents in leaves of rice plant.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at five developmental stages i.e. Pre flowering stage (P), Flowering stage (F), Seed formation stage (S) and seed mature stage (M) and pre harvesting stage (H) which corresponds to 67d, 86d, 105d, 120d and 132d of plant age respectively

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H
Control (H_2O)	5.07	3.78	2.51	1.75	0.29	4.49	3.64	2.65	1.74	0.14	4.64	3.35	2.91	1.59	0.34	5.8	3.36	2.61	1.59	0.24	4.46	3.64	2.11	1.03	0.43
ASA (250)	5.36	4.9	3.77	2.59	1.88	4.64	4.17	3.75	2.59	1.43	5.22	4.2	3.59	2.03	1.3	5.64	4.17	3.61	2.32	1.58	5.07	4.35	3.46	2.46	2.17
ASA (500)	5.51	4.78	4.2	3.61	2.9	6.52	4.64	4.52	3.45	2.03	4.78	4.06	3.78	2.9	2.18	4.78	4.46	3.74	2.9	2.75	5.22	4.88	4.22	3.88	3.62
SADH (150)	6.09	5.33	4.06	2.45	1.88	4.78	3.74	2.61	2.13	1.45	5.36	4.88	3.33	2.3	1.74	5.8	4.45	3.46	2.03	1.48	5.36	4.06	3.17	2.59	2.32
SADH (300)	5.65	5.36	4.36	2.17	1.75	6.38	4.35	3.38	2.45	2.17	4.93	4.35	3.62	2.74	2.32	5.07	4.35	3.07	2.9	2.45	5.07	4.35	4.17	3.19	3.03
NaDK (1000)	6.67	5.04	4.48	3.59	2.43	4.93	4.46	3.75	3.16	2.29	5.51	4.32	3.4	3.02	2.58	5.94	4.61	3.75	3.32	2.9	5.51	4.64	3.61	3.45	3.1
NaDK (2000)	5.36	4.78	4.06	3.46	2.29	4.49	4.13	3.09	2.74	2.23	5.22	4.93	4.06	3.88	2.43	6.23	5.51	3.91	3.03	2.29	5.65	5.51	4.14	3.58	3.32
LSD (P=0.05)	0.253	0.189	0.125	0.087	0.014	0.224	0.182	0.13	0.087	0.007	0.232	0.167	0.145	0.079	0.017	0.239	0.168	0.13	0.079	0.012	0.223	0.182	0.105	0.051	0.021

Table 3.20: Effect of pretreated Aromatic rice with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes soluble carbohydrate (µg/g fresh weight) contents in leaves of rice plant.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at five developmental stages i.e. Pre flowering stage (P), Flowering stage (F), Seed formation stage (S) and seed mature stage (M) and pre harvesting stage (H) which corresponds to 67d, 86d, 105d, 120d and 132d of plant age respectively

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H
Control (H_2O)	1.09	0.82	0.37	0.78	1.02	1.26	0.96	0.69	1.25	1.18	1.08	0.97	0.35	0.86	1.04	1.22	0.98	0.53	0.93	1.03	1.05	0.87	0.41	0.73	1.03
ASA (250)	1.39	0.92	0.84	0.97	1.28	1.22	0.91	0.73	1.07	1.34	1.28	1.06	0.77	1.05	1.2	1.27	0.96	0.67	1.07	1.22	1.2	0.95	0.72	1.02	1.12
ASA (500)	1.42	0.97	0.82	1.04	1.32	1.27	0.96	0.72	1.05	1.23	1.35	1.08	0.87	1.09	1.31	1.32	0.91	0.71	1.09	1.28	1.23	0.97	0.81	1.03	1.17
SADH (150)	1.28	0.79	0.61	0.96	1.24	1.24	0.92	0.79	1.02	1.2	1.38	1.13	0.97	1.15	1.23	1.23	0.92	0.84	1.04	1.21	1.22	0.91	0.75	1.09	1.14
SADH (300)	1.46	0.91	0.79	1.18	1.31	1.31	1.05	0.93	1.16	1.28	1.39	1.11	0.99	1.18	1.38	1.25	0.91	0.8	1.12	1.24	1.25	0.98	0.71	1.06	1.16
NaDK (1000)	1.53	1.25	1.03	1.29	1.44	1.58	1.34	1.04	1.32	1.49	1.56	1.37	1.17	1.32	1.49	1.36	0.99	1.07	1.14	1.35	1.24	1.07	0.95	1.06	1.23
NaDK (2000)	1.51	1.29	1.09	1.19	1.39	1.48	1.28	1.02	1.25	1.41	1.5	1.35	1.14	1.36	1.46	1.37	0.97	1.05	1.12	1.33	1.23	1.05	0.91	1.04	1.21
LSD (P=0.05)	0.054	0.039	0.051	0.039	0.051	0.061	0.045	0.034	0.051	0.059	0.054	0.048	0.017	0.043	0.052	0.061	0.045	0.026	0.046	0.051	0.052	0.043	0.02	0.036	0.051

Table 3.21: Effect of pretreated Aromatic rice with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes Insoluble carbohydrate (µg/g fresh weight) contents in leaves of rice plant.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at five developmental stages i.e. Pre flowering stage (P), Flowering stage (F), Seed formation stage (S) and seed mature stage (M) and pre harvesting stage (H) which corresponds to 67d, 86d, 105d, 120d and 132d of plant age respectively

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H
Control (H_2O)	10.4	11.1	11.8	12.4	7.14	7.16	9.4	12.9	14.1	4.77	11.6	11.3	8.97	13.4	6.67	8.87	9.89	12.7	13.9	5.97	9.64	10.3	11.8	12.9	5.81
ASA (250)	9.87	10.4	13.3	13.9	8.18	6.42	7.91	6.17	14.9	5.43	10.2	11.5	12.6	14.5	7.47	7.97	9.47	12.9	13.7	6.71	9.05	10.4	11.7	13.7	6.73
ASA (500)	10.1	10.9	11.6	13.1	9.31	7.47	9.41	13.4	13.9	6.93	10.6	12.1	13.7	14.9	8.49	9.37	10.1	12.8	13.8	6.74	10	11.3	12.7	13	7.22
SADH (150)	10	11.2	11.7	12	8.42	6.71	9.53	7.87	12.8	6.97	9.77	9.09	11.9	12.2	7.43	7.77	8.89	11.5	14.3	6.43	9.33	10.6	11.6	13.5	7.47
SADH (300)	11	10.4	6.98	14.2	9.18	7.41	9.97	14.2	14.9	8.49	10.9	12.9	13.4	13.9	7.71	9.89	11.3	14	14.6	7.65	10.1	10.9	12.1	13.5	8.34
NaDK (1000)	10	10.7	11.6	12.1	9.91	6.91	10.7	5.48	12.9	7.97	12	13.8	14	14.5	9.61	8.14	6.09	7.41	14.1	8.38	10.4	11.8	12.2	14.4	8.44
NaDK (2000)	10.8	11.2	12.2	13.3	9.69	7.65	10.9	13.9	14.5	8.92	11.8	13	13.7	14.3	8.49	9.67	12.9	13.9	14.7	7.24	10.6	11.2	12.9	13.5	7.53
LSD (P=0.05)	0.493	0.52	0.58	0.6	0.357	0.321	0.395	0.274	0.64	0.238	0.488	0.454	0.448	0.61	0.333	0.388	0.304	0.37	0.685	0.298	0.452	0.515	0.58	0.645	0.29

Table 3.22: Effect of pretreated Aromatic rice with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes catalase (µg/g fresh weight) contents in leaves of rice plant.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at five developmental stages i.e. Pre flowering stage (P), Flowering stage (F), Seed formation stage (S) and seed mature stage (M) and pre harvesting stage (H) which corresponds to 67d, 86d, 105d, 120d and 132d of plant age respectively

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H
Control (H_2O)	26.4	21.9	17.7	6.9	21.6	18.9	4.5	16.8	8.1	19.8	22.8	14.1	27.3	6	18	10.8	21.3	16.2	2.1	22.5	23.1	16.2	6.3	1.8	19.8
ASA (250)	21.6	22.8	21.6	6.9	19.5	17.1	18	6.6	8.7	22.8	12.6	21.6	48.6	8.7	18.3	11.5	18.6	12.9	0.9	19.8	55.8	27	9	3.6	18.9
ASA (500)	33.2	15.9	24	6.9	19.5	22.6	18.3	12	14.4	20.7	19.8	10.8	27.3	5.4	19.2	12.4	13.2	16.5	1.5	22.5	98.5	22.6	7.2	3	23.4
SADH (150)	31.6	25.8	20.7	7.8	20.7	12.6	13.5	6.6	8.4	23.7	25.6	13.5	41.1	8.4	17.1	3	13.5	12.9	0.6	20.4	53.5	27	9.9	9.6	21.3
SADH (300)	29.7	36	29.7	6.9	26.1	3	7.5	15.9	9.9	27.6	14.4	22.2	41.7	6	18	13.8	12.9	24	6.6	21.6	92.6	21.3	10.8	3.9	22.3
NaDK (1000)	38.4	12.6	18	8.4	29.1	33	18	7.2	9.9	22.5	10.8	16.2	33.6	5.4	45	34.8	26.7	14.1	6.3	23.4	169	57.3	16.2	9.6	21.3
NaDK (2000)	35.5	32.4	20.7	7.2	26.4	9.6	12	16.8	11.7	28.5	8.7	39.3	42.3	5.7	23.1	9.6	18.6	24.3	4.2	24	170	60	12	4.2	45.3
LSD (P=0.05)	1.08	0.63	0.885	0.345	0.975	0.15	0.225	0.33	0.405	0.99	0.435	0.54	1.365	0.27	0.855	0.15	0.645	0.645	0.03	0.99	1.155	0.81	0.315	0.09	0.945

Table 3.23: Effect of pretreated Aromatic rice with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes protease (µg/g fresh weight) contents in leaves of rice plant.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at five developmental stages i.e. Pre flowering stage (P), Flowering stage (F), Seed formation stage (S) and seed mature stage (M) and pre harvesting stage (H) which corresponds to 67d, 86d, 105d, 120d and 132d of plant age respectively

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H
Control (H_2O)	56.4	47.1	26.4	24.1	18.6	87.3	67.2	57.3	42	15.9	82.5	54.7	43.2	23.7	10.5	103	76.5	48.7	31.2	22.2	86.1	83.4	40.9	28.2	14.4
ASA (250)	59.1	49.2	34.9	29.8	22.2	80.2	68.4	56.4	33.6	20.4	135	105	93	54.7	36.7	133	112	75.3	51.8	38.5	101	81.4	64	32.1	21.3
ASA (500)	67.2	57.3	39.9	36.2	28.8	87	73.4	61	43.5	25.6	134	109	97.3	51.1	35.2	64.8	53.6	48.7	36.2	31.3	113	91.2	76.4	42.6	24.7
SADH (150)	58.8	54.3	38.1	30.1	25.5	80.7	59.1	53.4	38.7	19.2	60	49.2	34	44.7	27.7	134	106	86.2	59.4	39.6	120	90	66.9	49	36.6
SADH (300)	52.8	49.6	37.2	32.7	24.9	73.3	66	43.2	38.8	23.1	73	59.9	49.1	44.4	34.8	75.3	55.3	45.3	32	27.6	114	98.1	77.2	41.7	26.1
NaDK (1000)	71.1	59.3	52.7	48.2	44.4	84	83.1	67.2	52.9	39.9	105	98.4	72	61.5	37.2	80.4	65.1	54.3	50.8	45.9	91	73.5	65.7	48.8	43.2
NaDK (2000)	77.1	61.7	55.6	52.8	47.1	86.1	76.2	66	58.6	38.1	137	108	96	58.1	34.2	97.5	78.7	68.4	59.6	47.1	101	78.6	66.3	51.3	39
LSD (P=0.05)	2.64	2.355	1.32	1.205	0.93	3.665	3.3	2.16	1.68	0.795	3.65	2.46	2.16	1.185	0.525	4.02	3.255	2.435	1.56	1.11	4.305	3.675	2.045	1.41	0.72

Table 3.24: Effect of pretreated Aromatic rice with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes IAA Oxidase (µg/g fresh weight) contents in leaves of rice plant.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at five developmental stages i.e. Pre flowering stage (P), Flowering stage (F), Seed formation stage (S) and seed mature stage (M) and pre harvesting stage (H) which corresponds to 67d, 86d, 105d, 120d and 132d of plant age respectively

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H
Control (H_2O)	1.98	1.76	1.72	1.02	0.22	2.46	2.02	1.62	1.06	0.92	3.08	2.22	1.74	1.58	0.66	2.88	2.56	2.34	1.96	0.52	1.7	1.38	1.18	1.04	0.98
ASA (250)	2.72	2.42	1.84	1.74	1.66	3.6	3.08	2.76	2.24	1.46	2.48	2.16	1.88	1.55	1.26	3.24	3.06	2.42	1.76	1.54	2.78	2.36	1.94	1.76	1.44
ASA (500)	3.4	2.3	1.9	1.86	1.76	5	3.72	2.27	1.72	1.68	2.78	2.2	1.81	1.52	1.28	3.04	2.46	2.26	2.08	2.06	3.54	2.14	2.04	1.82	1.54
SADH (150)	2.5	2.2	2.14	1.74	1.62	3.64	2.88	2.47	1.92	1.74	3.2	3.18	2.61	1.86	1.58	3.28	2.34	2.12	1.72	1.66	2.78	2.62	2.44	1.72	1.56
SADH (300)	2.38	2.2	2.14	1.96	1.74	5.2	3.8	3.25	2.96	2.48	3.04	2.72	2.4	2.12	2.08	3.6	3.06	2.96	2.58	2.26	2.2	2.04	1.86	1.74	1.58
NaDK (1000)	3.42	2.96	2.94	2.46	2.38	4.92	3.14	2.98	2.44	2.52	3.8	3.44	2.5	2.38	2.2	3.5	3.22	3.02	2.8	2.4	3.46	3.12	2.8	2.68	2.48
NaDK (2000)	4.14	3.6	2.95	2.5	2.24	5.44	3.84	3.38	2.8	2.6	4.12	3.6	3.36	2.26	2.15	3.06	2.82	2.64	2.46	2.38	4.34	3.42	2.84	2.74	2.44
LSD (P=0.05)	0.099	0.088	0.086	0.051	0.011	0.123	0.101	0.081	0.053	0.046	0.124	0.11	0.087	0.076	0.033	0.144	0.117	0.106	0.086	0.026	0.085	0.069	0.059	0.052	0.049

Table 3.25: Effect of pretreated Aromatic rice with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes RNAse (µg/g fresh weight) contents in leaves of rice plant.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at five developmental stages i.e. Pre flowering stage (P), Flowering stage (F), Seed formation stage (S) and seed mature stage (M) and pre harvesting stage (H) which corresponds to 67d, 86d, 105d, 120d and 132d of plant age respectively

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																							
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																							
	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H
Control (H_2O)	3.94	3.76	2.88	0.32	2.74	3.8	5.78	1.96	0.82	2.36	2.7	3.2	4.2	0.34	1.8	3.7	5.48	6.34	1.4	2.16	2.36	4.2	4.52	0.68	2.44
ASA (250)	2.46	2.58	2.8	1.86	1.96	4	4.6	5.4	2.14	3.6	3.42	3.46	3.96	2.42	3.02	2.38	3.76	4.6	1.56	2.44	3.2	3.4	4.4	2.78	3.96
ASA (500)	3.48	4.48	5.08	2.12	3.62	4.7	5.62	6.42	3.54	3.92	3.78	4	4.82	2.16	3.14	3.54	5.38	6.14	1.86	2.84	2.84	4.7	5	2.14	3.34
SADH (150)	4.36	5.18	5.84	2.4	3.58	4.12	4.54	5	2.88	3.96	4.1	4.32	4.4	2.5	3.54	3.36	4.3	4.66	2.22	3	2.28	3.24	5.54	2.04	2.32
SADH (300)	3.42	4.74	5.28	3.36	3.94	4.8	5	6.52	3	4.64	3.54	4.22	4.3	2.34	3.24	4.6	5.1	7.8	2.44	3.62	3.06	4.12	4.9	2.5	2.78
NaDK (1000)	4.7	5.62	6.52	3.12	4.58	3.82	4.8	5.52	2.68	3.92	4.46	4.94	5.04	2.56	4.24	4.7	4.84	5.76	2.54	3.28	4.56	5.84	7.06	3.22	4.96
NaDK (2000)	3.98	4.4	6	3.9	4.06	4.32	4.64	6.08	3.04	4.04	4.58	5.08	5.44	2.54	3.24	4.56	5.02	6.48	2.5	3.94	3.62	4.66	5.04	3.54	4.44
LSD (P=0.05)	0.123	0.129	0.14	0.016	0.098	0.19	0.227	0.098	0.041	0.118	0.135	0.16	0.198	0.017	0.09	0.119	0.188	0.23	0.07	0.108	0.114	0.162	0.22	0.034	0.116

Table 3.26: Effect of pretreated Aromatic rice with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes superoxide dismutase (µg/g fresh weight) contents in leaves of rice plant.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at five developmental stages i.e. Pre flowering stage (P), Flowering stage (F), Seed formation stage (S) and seed mature stage (M) and pre harvesting stage (H) which corresponds to 67d, 86d, 105d, 120d and 132d of plant age respectively

Pre-treatments (µg/ml)	DIFFERENT VARIETIES OF AROMATIC RICE																								
	MOHANBHOG (M)					KHEMTI (H)					MASINO BASMATI (B)					MUSLI (S)					KALONUNIA (K)				
	DAYS OF DIFFERENT PRETREATED PERIODS																								
	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H	P	F	S	M	H
Control (H_2O)	3.27	1.29	0.9	1.2	0.24	4.47	1.2	0.57	0.81	0.12	2.4	1.5	0.78	0.91	0.24	1.89	1.39	0.96	1.06	0.06	1.71	1.02	0.66	0.69	0.18
ASA (250)	4.86	1.92	1.13	1.81	1.69	1.83	1.63	1.47	1.78	1.3	3.3	1.35	1.16	1.81	1.21	4.53	1.92	1.26	3.36	1.6	3.27	1.02	1.26	1.6	1.48
ASA (500)	2.61	1.89	1.29	4.5	1.6	1.98	1.75	1.65	1.9	1.36	1.74	1.08	0.96	1.22	1.48	2.22	1.75	1.57	1.81	1.42	2.97	1.09	0.96	1.86	1.51
SADH (150)	2.67	1.26	1.06	2.69	1.66	4.71	1.62	1.27	1.84	1.39	4.77	1.81	1.75	2.47	1.36	5.07	1.86	1.72	3.3	1.99	3.39	1.96	1.38	1.54	1.44
SADH (300)	3.06	1.62	1.39	5.13	2.06	4.23	1.38	1.14	1.9	1.49	4.38	1.71	1.65	2.05	1.33	5.19	1.92	1.57	3.74	2.19	3.33	1.59	1.41	3.54	2.45
NaDK (1000)	5.13	1.83	0.6	4.36	3.14	4.47	1.83	1.47	3.96	2.42	5.16	1.32	1.08	3.2	2.54	4.89	1.99	1.42	3.09	2.33	4.4	1.8	1.26	3.66	2.16
NaDK (2000)	5.88	1.65	0.9	4.86	2.93	3.87	1.89	1.62	3.02	2.6	5.28	1.5	1.2	3.14	1.96	5.7	1.95	1.63	3.2	2.09	3.84	1.93	1.29	3.69	1.81
LSD (P=0.05)	0.13	0.063	0.03	0.06	0.012	0.091	0.06	0.028	0.04	0.006	0.12	0.066	0.039	0.045	0.012	0.094	0.069	0.048	0.053	0.003	0.085	0.051	0.033	0.034	0.009

Table 3.27: Effect of seed pretreatment with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on yield attributes per panicle of aromatic rice types Kalonunia, Mohanbhog, Khemti, MasinoBasmati and Musli.

Seeds were pretreated with test solution or distilled water for 6h and then sundried. This was repeated three times in close succession and the seed lots were kept in 95% RH. Plants were raised from accelerated aged in the experimental field and data were recorded after harvest

Pre-treatments (µg/ml)	YIELD ATTRIBUTES									
	DIFFERENT VARIETIES OF AROMATIC RICE									
	MOHANBHOG		KHEMTI		MASINO BASMATI		MUSLI		KALONUNIA	
	Seeds wt. / Panicle	1000 seeds wt. (g)	Seeds wt. / Panicle	1000 seeds wt. (g)	Seeds wt. / Panicle	1000 seeds wt. (g)	Seeds wt. / Panicle	1000 seeds wt. (g)	Seeds wt. / Panicle	1000 seeds wt. (g)
Control (H_20)	4.824	24.12	5.641	22.564	5.88	23.52	4.674	23.37	5.492	19.476
ASA (250)	5.693	28.465	6.885	27.54	6.938	27.752	6.182	30.91	7.179	21.537
ASA (500)	5.838	29.19	6.983	27.932	7.067	28.268	6.383	31.915	7.415	22.245
SADH (150)	5.556	27.78	6.593	26.372	6.753	27.012	5.989	29.945	7.755	23.265
SADH (300)	5.784	28.92	6.735	26.94	6.882	27.527	6.288	31.44	7.683	23.049
NaDK (1000)	6.966	34.83	7.937	31.748	7.873	31.492	6.985	34.925	8.292	24.876
NaDK (2000)	6.901	34.505	7.683	30.732	7.698	30.792	7.078	35.39	7.849	23.535
LSD (P=0.05)	0.241	1.206	0.282	1.128	0.294	1.176	0.233	1.168	0.274	0.973

Table 3.28: Effects of seed pretreatment with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes in plant height (cm) before and after shifting of seedlings of aromatic rice types Kalonunia, Mohanbhog, Khemti, MasinoBasmati and Musli.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at before shifting of rice plants in (0-5, 15 and 25 days) and after shifting of rice plants in (45, 60, 75, 90, 105, 120 and 135 days) of plant age respectively.

Different varieties of aromatic rice	Pre-treatments (µg/ml)	Plant Height (cm)									
		Days of developmental Stages									
		Before shifting of plant			After shifting of plant						
		0-5	15	25	45	60	75	90	105	120	135
1. KALONUNIA	Control	NE	5	12.5	17	26	55.8	85.8	91.44	108.4	118
	ASA 250	NE	5.5	13.5	18	27	61.5	90.8	112.5	120.4	122.8
	ASA 500	NE	5.8	13.8	18.4	30	61.6	91.65	115.9	122.5	123
	SADH 150	NE	5	14	17.8	26.4	58.8	91.2	117.9	121	121
	SADH 300	NE	5.5	13.8	17.5	26.5	60.9	91.65	116.6	120	122.9
	NADK 1000	0.5	8.9	15	22.5	30	62	92.5	118.9	121.9	123
	NADK 2000	NE	8	14.5	21.8	28	61.5	91.8	118	121	123
	LSD(P=0.05)	NS	0.25	0.62	0.85	1.3	2.79	4.29	4.57	5.42	5.9
2. MOHANBHOG	Control	NE	5.5	12.8	21.5	30.5	61	91.2	118	120	121
	ASA 250	NE	5.6	15.2	25	35	61.4	91.57	120	123	123
	ASA 500	NE	5.8	15	27.8	41	91.9	112	122	124	124
	SADH 150	NE	6.6	13.6	22.2	31.2	62	92	118.9	122	122
	SADH 300	NE	6.9	14.7	24.6	33.8	65.9	95.9	121.4	123	123
	NADK 1000	2	7.5	15	26	41	91.4	118.9	122.6	124	124.6
	NADK 2000	2	7	15	24.8	38.9	91	118	122	123	124.2
	LSD(P=0.05)	NS	0.27	0.64	1.07	1.52	3.05	4.56	5.9	6	6.05

3. **KHEMTI**	**Control**	NE	6.6	13.7	21	30.6	60.5	91	108	116	121
	ASA 250	NE	8.6	15.6	20	32.5	60.4	91.5	120	123	123
	ASA 500	NE	9	17.8	23.4	34	63.5	93.5	122	125	125
	SADH 150	NE	7	12.8	20.6	31.8	61.8	92.9	120.5	123	123
	SADH 300	NE	8	14.2	22.5	34.2	63	94	122.7	124	124.6
	NADK 1000	4.2	9.2	16.6	26.8	34	71.8	96.2	123	125	127
	NADK 2000	3	9.1	16.8	26.5	30.8	70.5	96	122	125.5	126
	LSD(P=0.05)	**NS**	**0.33**	**0.64**	**1.03**	**1.53**	**3.02**	**4.55**	**5.4**	**5.8**	**6.05**
4. MASINOBASMATI	**Control**	NE	6.5	15	21	39.5	61.8	90.5	112.5	117	119
	ASA 250	NE	8	17	22.5	38	71.6	91.5	116.6	121	121
	ASA 500	NE	9.2	17.8	25.8	43	86.8	108	120	120.5	122
	SADH 150	NE	8.4	15.5	25.5	41.9	84.8	113	120	121.8	122
	SADH 300	NE	9	16.4	27	43	90.9	118	121.2	123	123
	NADK 1000	NE	7.6	15.6	28.8	43.2	91.6	118.9	122	123	123.8
	NADK 2000	NE	7.8	15.8	28	42	91.5	108	121.5	122.1	123
	LSD(P=0.05)	**NS**	**0.32**	**0.75**	**1.05**	**1.9**	**3.09**	**4.52**	**5.62**	**5.85**	**5.95**
5. MUSLI	**Control**	NE	6.2	12	20.3	34	60.2	90.8	120	121	121
	ASA 250	3	8	17	22	37.2	60.1	91.6	121.2	122	122
	ASA 500	2.7	8.5	17.6	25	38	62	93	122.5	123.2	123.8
	SADH 150	2.3	5.8	12.5	20	37.8	55.8	85.9	106.6	120.5	121.5
	SADH 300	2.2	6.5	13.4	22.8	38	57.2	88.6	116	122	122.6
	NADK 1000	3	7.6	14.4	25.5	39.4	91.2	121	122	122.5	124
	NADK 2000	3	7.9	14.2	24.8	41	91.5	120	122	123	123
	LSD(P=0.05)	**NS**	**0.31**		**1**	**2.7**	**3**	**4.54**	**5.33**	**6.05**	**6.05**

NE: NO EMERGENCE OF SEEDS

NS: NOT SIGNIFICANT

Table 3.29: Effects of seed pretreatment with Ascorbic Acid (ASA, 250 and 500µg/ml), Succinic Acid Dehydroxide (SADH, 150 and 300µg/ml) and Sodium Dikegulak (NaDK, 1000 and 2000µg/ml) followed by 360 days of accelerated ageing treatments of the seeds on changes in internodal elongation (cm) and stem circumference (cm) of aromatic rice types Kalonunia, Mohanbhog, Khemti, MasinoBasmati and Musli.

Treated seed lots of five varieties **Mohanbhog, Khemti, MasinoBasmati, Musli and Kalonunia** were sown in the experimental field. Data were recorded at 45d, 60d, 75d, 90d, 105d, 120 d and 135 days of plant age respectively.

Rice varieties	Pretreatment (µg/ml)	Days of Developmental Stages													
		Internodal elongation							Stem circumference						
		45	60	75	90	105	120	135	45	60	75	90	105	120	135
1. KALONUNIA	Control	6	13	18.5	24	30.4	30.5	29.6	2	3	3.5	3.2	3.2	3	3
	ASA 250	7	14	19	24.5	30.5	30.6	30.6	2.1	3.5	4.2	4	3.5	3.4	3.4
	ASA 500	6	14	19	24	30	30.2	30.5	2.1	3.5	4.2	3.9	3.5	3.3	3.3
	SADH 150	6.6	14	21	29.6	30	30	30	2.1	4	4.2	4	3.5	3.4	3.4
	SADH 300	6.5	13	19	24.5	30	30	30	2.4	4	4	3.6	3.4	3.3	3.3
	NaDK 1000	8	15	21.8	30	30.5	30.9	31	2.5	4.1	4.2	4	3.7	3.5	3.5
	NaDK 2000	7	15	21.6	30	30.5	30.8	31	2.5	4.1	4.2	4	3.5	3.5	3.5
	LSD(P=0.05)	0.3	0.65	0.92	1.2	1.5	1.5	1.5	0.1	0.15	0.17	0.16	0.16	0.15	0.14
2. MOHANBHOG	Control	7	15	19	23.5	31.2	31.5	30	2.3	3	3.5	3.2	3	2.8	2.8
	ASA 250	7	14	19	24	30.6	31	31.2	2.5	3.4	3.5	3.5	3.3	3.3	3.3
	ASA 500	6	13	19	23.8	30.4	30.8	31	2.5	3.5	3.5	3.3	3.1	3	3
	SADH 150	7	14	19.5	24.4	29.6	30	30	2.5	3.3	3.3	3.3	3.2	3.1	3.1
	SADH 300	7	14	19.2	23.9	30	30	30	2.5	3.5	3.5	3.3	3.2	3.2	3.2
	NaDK 1000	8	15	22.8	24.6	30.5	30.5	31.4	3	4	4.5	4.3	3.8	3.6	3.6
	NaDK 2000	8	15	22	24.5	30	30.56	31	3	4	4.5	4	3.8	3.5	3.5
	LSD(P=0.05)	0.35	0.65	0.95	1.17	1.5	1.5	1.5	0.11	0.15	0.17	0.16	0.15	0.14	0.14

4.KHEMTI	**Control**	6	14	28	30.5	31	31	31	2	3	3.5	3.4	3.2	3	3
	ASA 250	6.8	14	29	30.4	31	31	31	2	3.5	3.5	3.4	3.3	3.3	3.3
	ASA 500	6.5	14	29	30	30.8	31	31	2.5	4.5	4.5	4.3	4.3	4	4
	SADH 150	7.5	14	29.4	30	30.5	30.8	30.8	2	4	4	3.9	3.5	3.3	3.3
	SADH 300	7	14	29	30	30.6	30.6	30.6	2.3	4.3	4.2	4	4	4	4
	NaDK 1000	8	15.5	30	31	32	32	32	3	4.5	4.5	4.3	4.2	4	4
	NaDK 2000	8	15	30	31.5	32	32	32	3	4	4.3	4.2	4.2	4	4
	LSD(P=0.05)	**0.3**		**1.4**	**0.15**	**1.52**	**1.53**	**1.53**	**0.1**	**0.15**	**0.17**	**0.17**	**0.16**	**0.15**	**0.15**
4. MASINOBASMATI	**Control**	8	17	28	30	30	30	30	2.3	3	3.7	3.5	3.2	3.2	3.2
	ASA 250	9	18	30	30.8	31	31.5	31.5	2.1	3	3.8	3.5	3.4	3.4	3.4
	ASA 500	8	18.4	30.4	30.4	30.5	30.8	30.8	2.9	4.5	4.3	4.3	4.1	4.1	4
	SADH 150	8	18	30	30	30	30.5	30.6	2.1	3.3	3.5	3.5	3.3	3.2	3.2
	SADH 300	8	18.5	28.8	30	30	30.6	30.6	2.4	3.7	3.5	3.5	3.4	3.3	3.3
	NaDK 1000	9.5	21	30	30	30.5	31.6	32	2.8	4.5	4.3	4.3	4.1	4	4
	NaDK 2000	9	21	30	30.5	30.8	31.4	32	2.6	4	4.3	4.1	4	4	4
	LSD(P=0.05)	**0.4**	**0.85**	**1.4**	**1.5**	**1.5**	**1.5**	**1.5**	**0.10**	**0.15**	**0.17**	**0.17**	**0.16**	**0.16**	**0.16**
5. MUSLI	**Control**	5	9.5	16.5	20.5	28.5	30	30	2.2	2.2	3.4	3.5	3.5	3.3	3.3
	ASA 250	5.5	10.5	18	29.6	29.6	30	30.7	2.9	4	4	3.7	3.7	3.5	3.5
	ASA 500	5.8	10	15	30.5	28.9	30.4	30.5	2.8	4.1	4.3	4.3	4	4	4
	SADH 150	5.4	9	15.2	30.3	28	28.5	30.6	2.1	3.4	4	3.8	3.5	3.5	3.5
	SADH 300	5.5	9	15	30	28.8	29	30.6	2.1	3.8	4.3	4.5	4.2	4	4
	NaDK 1000	6	10.8	18.2	30	30	30.5	31.8	2.6	4.2	4.5	4.5	4.3	4.3	4.3
	NaDK 2000	6	10.6	18	30	29.6	30.5	31.7	2.8	4	4.3	4.5	4.4	4.2	4.2
	LSD(P=0.05)	**0.25**	**0.45**	**0.75**	**1.02**	**1.4**	**1.5**	**1.5**	**0.11**	**0.11**	**0.17**	**0.17**	**0.17**	**0.16**	**0.16**

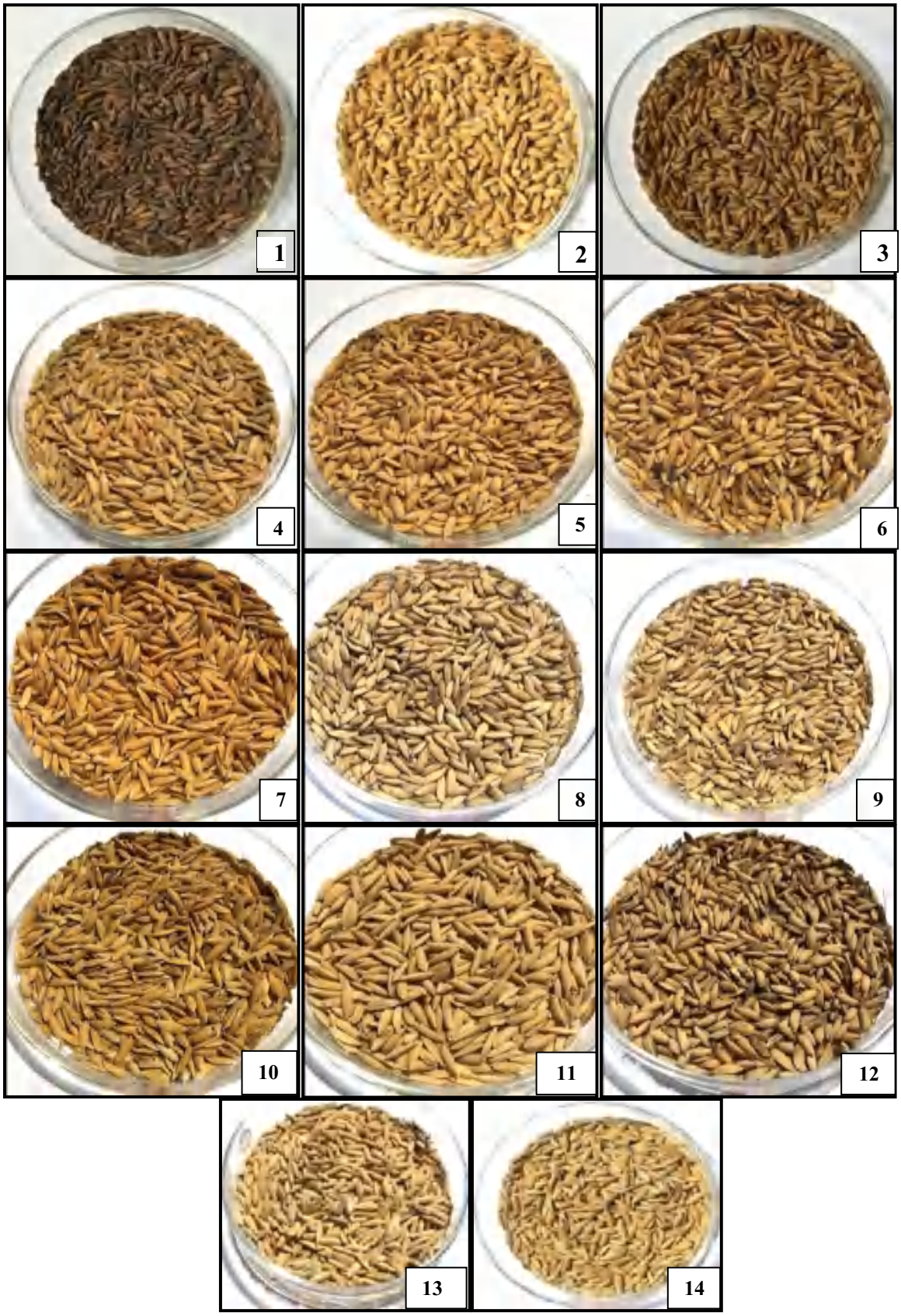


Fig.5. 14 different varieties of local aromatic rice seeds.

1. Kalonunia, 2.Addey, 3.Mansari, 4. Kattaka, 5.Rajabahara, 6.Mohanbhog, 7.Khemti, 8.Masinobasmati, 9.Krishnabhog, 10.Musli, 11.Dudhraj, 12.Fulpaty, 13.Arya, 14.Birimful.

Fig.6. Field preparation procedure.

Fig.7. The field was adequately puddled with bullocks.

Fig.8. Rice seeds from each treatment were manually spread on the prepared flooded field.

Fig.9. Seed emergence in the field.

Fig.10. The first leaf breaks through the coleoptiles.

Fig.11. Rice seedlings growth and development prior to transplantation.

Fig.12. After transplantation of uprooted rice seedlings (Left).

Fig.13. Rice plants increase in length and tillering after transplantation (Right).

Fig.14. Panicle initiation and Booting stage of rice plants.

Fig.15. Flowering stage of Kalonunia rice plants.

Fig.16. Flowering stage of pretreated rice plants.

Fig.17. Flowering stage of pretreated rice plants.

Fig.18. Flowering stages of pretreated rice plants.

Fig.19. Development of milk grain stages of rice plants.

Fig.20. Development of milk grain stages of rice plants.

Fig.21. Development of milk grain stages of rice plants.

Fig.22. Seeds development stages in pretreated rice plants.

Fig.23. Fully developed seeds of pretreated rice plants.

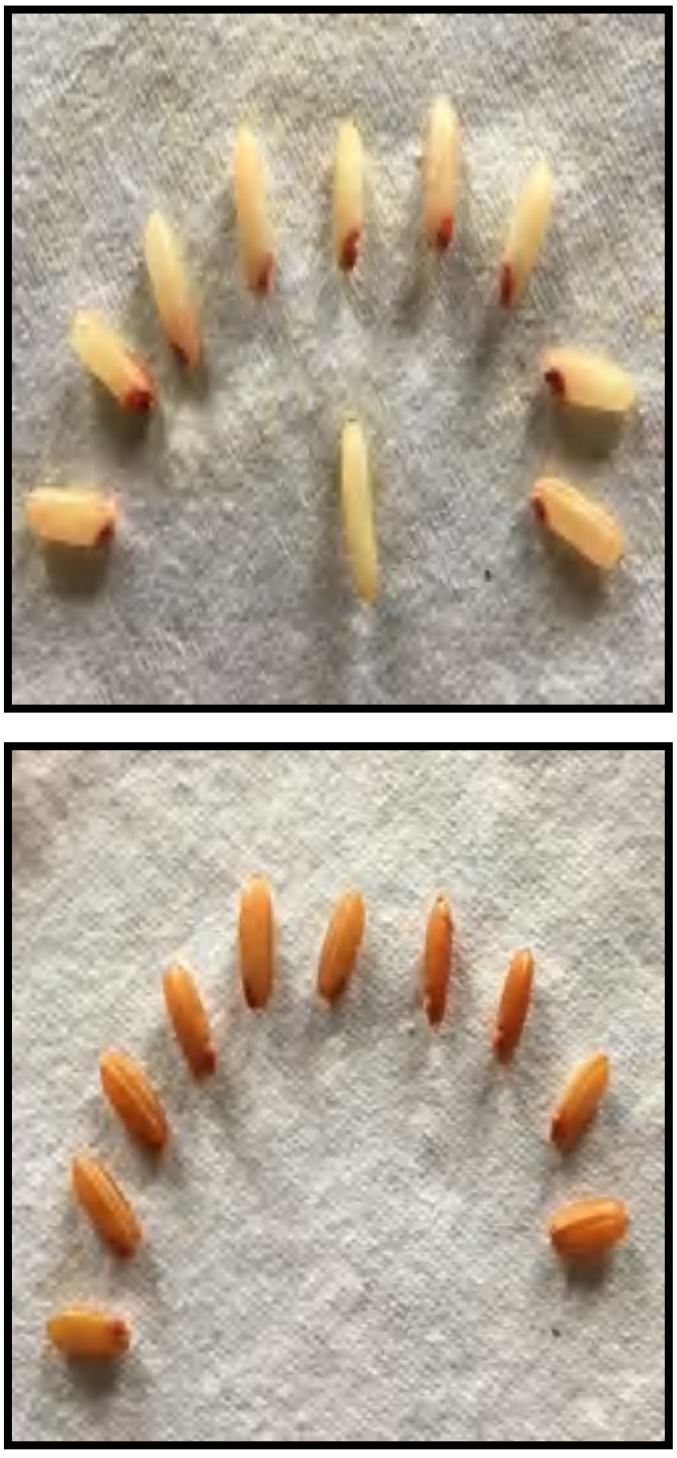


Fig.24. TTC stained rice seeds samples.

Fig.25. A. Rice seeds size before cooking. B. Elongation of rice seeds after cooking

Fig.26. Germinating rice seeds in Petri plate showing emergence of radicals and coleoptiles.

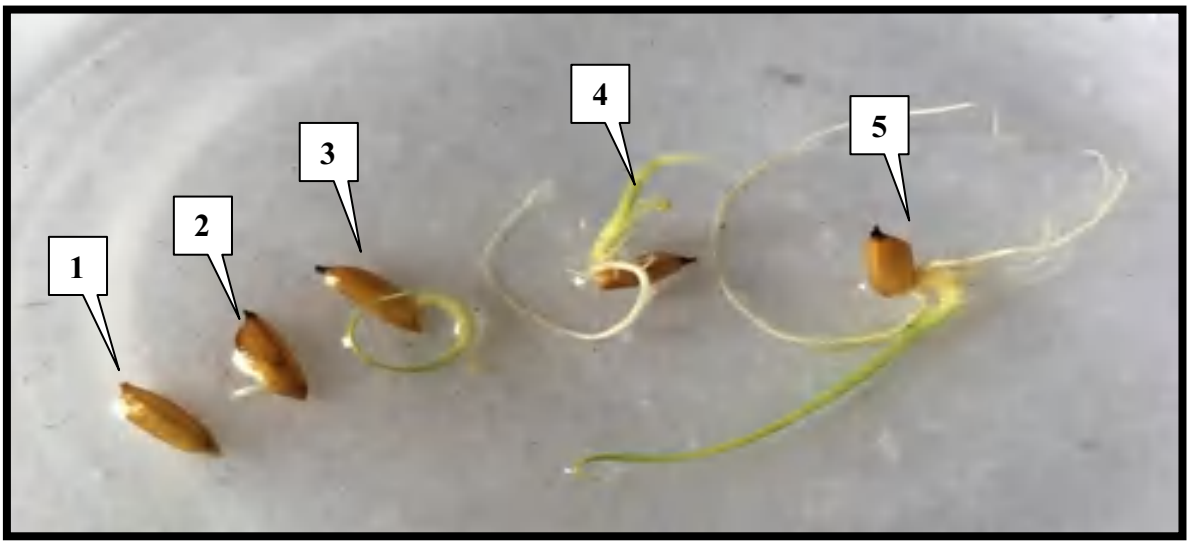


Fig.27. Developing stages of rice seeds in laboratory.

1. **Rice seed, 2. Emergence of radical from germinating seed, 3. Development of coleoptiles, 4. Development of root and shoot, 5. Development of roots and shoot of rice plant.**

Fig.28. Development of rice seedlings in laboratory.

Fig.29. Experimental field of rice cultivation with worker.

Chapter V

Discussion

5. DISCUSSION

During seed storage, seed degradation is a normal, catabolic, permanent, and internal degenerative programed phenomenon. The seed quality, vigour, and viability in the process of ageing or adverse ecological conditions are translated as a loss. However, the deterioration rate of seed depends on the different types of seed species due to the seed moisture content and storage temperature, an increment or decline in one or the other leading to an expeditious or delayed deterioration process. The physical and chemical efficacy of seed degradation has recently been widely exposed (Kapoor, *et al*., 2010; Mahjabin, *et al*., 2015; Somasundaram, *et al*., 2017; Wang, *et al*., 2018; Fenollosa, *et al*., 2020). Numerous physiological and biochemical manipulative strategies have been invented by seed technologists to eradicate such climatic as well as biotic hazards that are conducive to earlier deterioration of stored seeds. There are a few reports that hydration-dehydration treatment, as well as chemical treatment of seeds of various natures, such as salts, phenols, vitamins, organic acids, antioxidants, essential oils, plant growth regulators, retardants, and so on, can significantly impact the viability status of the seeds (Bhattacharjee *et al*., 1984, 1993; Rai, 2000; Draganic and Lekic, 2012; Ojha, 2014; Lama, *et al*., 2016; Sasikala, *et al*., 2018; Palit, *et al*., 2019; Pati, 2020).

Degradation of seeds follows a convoluted contour that diversifies with the wide classes of desiccation tolerant, recalcitrant, and even seed species from various plant taxa. Reduced germinability is thus, like the difficult senescence processes of higher plants, the most widely-recognized criteria for the complex mechanism of seed deterioration. A number of studies have been carried out to evaluate loss of vigour based on ageing physiological consequences (Siddiqui, *et al*., 2011; Sarika, *et al*., 2013; Adeboye, *et al*., 2015; Shruthi, *et al*., 2018; Chan, *et al*., 2019; Nigam, *et al*., 2019; Laxmi, *et al*., 2021). One of the fundamental and important technologies of accelerated ageing is exposing seed to elevated temperatures and high relative humidity.It was recommended that a cold and accelerated ageing test be used to determine seed vigour, and that the results be compared to real field emergence (Nirja *et al*., 2018).

Owing to the lack of commercial seed substitutes, the majority of rice farmers have used a casual seed scheme for many years, relying on their self-conserved vulnerable and low-vigor rice seeds each year. This could decrease rice production overall, but a proper seed

vigour test could avoid this problem and increase production by substituting high-vigor seeds. Because of the large quantities of rice seed required, seed production improves dramatically, but there is a chance of poor-quality seed remaining in the system. There must also be an effective quality management framework for rice seeds in order to guarantee seed quality, which includes seed vigour determination. Global warming has resulted in unfavourable climatic conditions, which can affect rice seed vigour and, as a result, reduce seed life in surrounding storage conditions. In all phases of rice production, such as seedlings, planting, flowering, harvesting, and so on, farmers are unable to anticipate ideal climatic conditions. Farmers can withstand the negative effects of climate change by using the most vigorous seeds. Planting rice seed varieties susceptible to ageing could withstand unfavourable conditions in the field, which would have a stable population of seedlings and plants.

The accelerated ageing test has been effectively utilised in anticipating the vigour and storage capability of various crop species under a wide range of storage conditions (Delouche and Baskin, 1973). The accelerated ageing test specified the vigour of Mungbean seeds at 42°C for 72 hours, corn at 45°C for 72 hours, soybeans at 42°C for 48 hours, and carrot, tomato, safflower, onion, gram, pea, sunflower, beetroot seeds, onion seeds, etc. as more efficient (Dutra, *et al*., 2004; Silva, *et al*., 2006; Pati, *et al*., 2011 & 2015; Vijay, *et al*., 2015; Patil, *et al*., 2018; Luciana, *et al*., 2019; Jangjoo, *et al*., 2020; Chaurasia, *et al*., 2021; Bhattacharjee, *et al*., 2021). Investigation into the accelerated ageing test for rice seed storability noticed accelerated ageing at 40°C and 100% RH for 1–15 days could anticipate rice longevity under storage conditions (Lee *et al*., 2018). Cold and accelerated ageing tests with implied field emergence could be applicable for rice seed vigour tests (Baek *et al*., 2018; Nirja *et al*., 2018). Studies have shown that artificial ageing tests are an effective technique to contemplate rice seed vigour rather than natural ageing (Kapoor, *et al*., 2011; Wang, *et al*., 2012; Bijanzadeh, *et al*., 2017; Henga, *et al*., 2019; Zhou, *et al*., 2020; Alahakoon, *et al*., 2021).

Analysis of the present investigation was performed with the five different varieties of aromatic rice seeds (MohanBhog, Khemti, MasinoBasmati, Musli, and Kalonunia) with the view to assessing their viability status under an unfavourable storage environment by analysing some putative and reliable biochemical parameters. In the current exploration, accentuation was laid on prolongation of seed viability under storage by utilising pre-treating chemicals Ascorbic acid, SADH and Na-dikegulac of definite concentrations, which is by all

accounts promising in such manner in a couple of recent investigations (Chhetri, *et al*., 1992; Rai, *et al*., 1995; Maity, *et al*., 2000; Bhattacharjee, *et al*., 2006; Ojha, 2013; Lama, *et al*., 2016; Moori, *et al*., 2017; Bhattacharjee, *et al*., 2018; Pati, *et al*., 2019; Tamang, *et al*., 2020; Kanp, *et al*., 2021). The prospect of NaDK, ASA and then SADH respectively as beneficial chemical pre-treatment for maintaining vigour and the viability of seeds have been explored based on the various knowledge available and findings obtained until now during this study.

According to findings in this experiment, under long-term accelerated ageing starting from 0-, 90-, 180-, 270-, and 360-days, seed vigour decreased as ageing time increased, indicating a negative relationship. Seed vigour was drastically impaired with an increase in days of treatment. The data showed that a high relative humidity treatment increased the forced ageing process and this increase of leaky substances reduced the levels of protein, carbohydrate, amino acids as well as enzyme activities, which was considerably checked by seed pretreatment with NaDK, ASA, and SADH, respectively **(Figs 1, 2 & 3)**. However, a significant alleviation of the injurious effect was noted best in seeds that underwent pretreatment with NaDK, then in ASA and then in SADH, respectively.

The findings show that seed degradation and seed membrane damage occur as a result of accelerated ageing. Seed degradation occurs through a series of processes starting with biochemical event chains, mostly impaired liver and biosynthetic reactions, and resulting in the loss of several seed output characteristics, a decreased rate of germination, decreased field outbreaks, a rise in the number of seedlings that are unhealthy, and, ultimately, the death of seeds. Some of the most serious deterioration-causing physiological and biochemical activities are membrane degradation, changes in the cell's chemical components, decreased metabolic activity, free radical disruption, chromosome aberrations, and degradation of functional structures. reduced field emergence, a rise in irregular seedlings, and eventually seed death. Membrane degradation, enzyme changes, changes in cell chemical constituents, decreased metabolic activity, free radical disruption, chromosome aberrations, and degradation of functional structures are just a few of the significant physiological and biochemical events that occur as a result of deterioration. Seed deterioration is associated with chromosome aberrations and DNA injuries; impairment of RNA and protein syntheses; altered enzymes; nutrient stores; and membrane integrity depletion (Wang *et al*., 2012; Pati *et al*., 2014; Mahjabin *et al*., 2015; Chauhan *et al*., 2019; Ebone *et al*., 2019).

Work on the decline in germinability and viability has backed up the idea that reduction in integrity and the lesion incidence of the membrane may play a significant role in seed deterioration. Cellular constituents undergo permanent chemical and structural changes as a consequence of viability failure. Reduced membrane fluidity, altered DNA folding, protein elasticity loss, and increased cellular matrix brittleness are all structural changes associated with oxidation. Molecule oxidation results in small, readily diffused molecules with reagent carbonyl or nitrogen groups, or adducts that encourage cross-linkage or consequent breakdown of advanced glycation products between proteins, carbohydrates, and nucleic acids across cells. Phospholipids and phosphatidyl choline levels drop as seed germination decreases, causing membrane integrity to deteriorate (Walters *et al*., 2010). In an accelerated ageing environment of seed germination drops, the depletion of membrane integrity of phospholipids and phosphatidyl choline occurs. According to some studies, the ability of seeds to quickly reorganise their membrane for efficient germination is because of the desiccated rehydration of their tissues. Many studies have suggested that the membrane state of the germinating embryo is a significant degradation factor (Kibinza *et al*., 2006; Kapoor *et al*., 2011; Pati *et al*., 2019; Ebone *et al*., 2020).

The supporting references presented on membrane integrity and chemical pretreatment of seeds; thus, significantly less leaching of soluble carbohydrates and amino acids from the used chemical-pretreated seeds is suggestive of the fact that NaDK, SADH, and then ASA, respectively, rendered the seeds tolerant against storage deterioration in an unfavourable environment by retaining the integrity of the seed membrane. As seen in this study, forced ageing-induced membrane damage may be a factor in their rapid deterioration during storage. The supportive references to membrane integrity and chemical pretreatment of seeds, and substantially less leaching of soluble carbohydrates and amino acids of the chemically-treated seeds used, show that NaDK, SADH, and ASA have been tolerant to storage deterioration by preserving seed integrity in an unfavourable environment. Changes in a variety of biochemical parameters examined here, which are considered reliable indices of seed vigour, help the efficacy of NaDK, SADH, and ASA in maintaining the health of five aromatic rice seeds during the accelerated ageing time. The levels of protein **(Table 1.7)**, RNA **(Table 2.11)**, and insoluble carbohydrates **(Table 2.10)** steadily decreased in control samples during rapid ageing, with ageing length correlated with a proportional change in metabolism within seed kernels, but this pattern was significantly delayed by the pre-treating

chemicals. Again, with the increase of accelerated ageing, a drastic reduction of protein and insoluble carbohydrates was noted. Here also, the pre-treating chemical efficiently relieved the deleterious effect of forced ageing treatment. Among the chemicals used, Na-dikegulac arrested the alarming rise in internal soluble carbohydrate level compared to the other chemicals used, ASA and SADH, as observed in experiments.

Seed degradation under storage is accelerated or postponed, determining the life period of a given seed species, resulting in a loss of vigour, viability, and subsequent decay of seeds, eventually leading to seed death. Despite the fact that both treated and control samples of aromatic rice seeds deteriorated, the catabolic processes inside the treated seeds remained somewhat subdued, making them more resistant to unfavourable storage conditions. According to studies, certain essential cellular components are lost during seed maturation. During the course of seed deterioration, there has been a reported increase in soluble compounds, amino acids, and nucleic acids. As a consequence, the findings obtained are consistent with the observations made (Anderson and Gupta, 1986; Bhattachharjee, 2005; Kim, *et al*., 2006; Kapoor, *et al*., 2011; Ojha, *et al*., 2012; Pati, *et al*., 2015 & 2019).

The analyses of control samples showed that both dehydrogenase activity and the percent of TTC-stained seeds were sharply decreasing and that an increase in ageing trials revealed sudden decreases in enzyme activity and seed species TTC stainability. The preprocessing chemical partly prevents the adverse impact and effectively alleviates the adverse effects of accelerated ageing substantially. The function of dehydrogenase is usually used as a valid seed reliable index (Patil *et al*., 2015; Pati *et al*., 2018; Chauhan *et al*., 2019). There are also reports that as seeds age, they lose vigour, which is evaluated by counting the percentage of TTC-stained seeds and/or by observing the pattern of TTC-staining that appears as As a seed-age, they often lose their vigour, which is assessed by counting TTC-stained seed percentages and/or by observing the TTC-stained pattern that is seen as deep red or erratic red patches on the seeds depending on their viable condition (Halder, 1981) **(Fig. 24)**. In this study, despite the accelerated ageing process, chemical seeds pretreated caused a mitigation of the deleterious consequences of ageing and kept the plants more vigorous than controls and influenced metabolically.

The detailed work was performed with the pretreated five varieties of aromatic rice seeds that experienced accelerated ageing for 0-, 90-, 180-, 270-, and 360-days in this

inquiry. The test chemicals NaDK, SADH, and ASA each have a storage enhancement property, as evidenced by the cessation of seed germination loss and field emergence. It has been found that in control samples under accelerating ageing, percentage germination and field emergence **(Table 1.6)** have been adversely affected, but the inhibitor effects have been somewhat reversed in pretreatment chemical products. The assessment of low seed vigour is seen as a significant observable criterion for decreased germination and a slower rate of germination. Seedling establishment is affected by reduced vigour as treatment duration increases (Rai, 2000; Kanp *et al*., 2009; Dey *et al*., 2012; Pati *et al*., 2015; Mangena *et al*., 2019).There have also been reports of chemical improvements in seed germination and metabolism, and this finding conforms to the observations recorded. Accelerated ageing has detrimental effects on the germination of seed and seed development in chemicals like NADK, SADH, and ASA. The seeds seem to be hardening under unfavourable storage conditions.

The reduction of DNA and RNA **(Table 2.11 & 2.12)** levels, soluble carbohydrate **(Table 2.9)** and free amino acids (Table), subdued activities of dehydrogenase, amylase and catalase enzymes **(Table 1.8)**, enhanced activities of oxidase and protease enzymes (**Table 2.14)** in seed cotyledons and the relieving action and efficacy of chemicals NaDK, SADH, and ASA pretreatment are indicative of storage potentiaton property and quality property of these chemicals. The chemical induced alleviation of the deleterious ageing impacts on the overall development and metabolism of plants, which indicates the effectiveness of the pretreatment chemical. When seeds were chemically pretreated and tested for the potential of field emerging, higher protease levels **(Table 3.23)**, and activity of catalase **(Table 3.22)** and peroxidase enzymes **(Table 3.26)** compared to controls, the plants were also shown to have significantly better ability and performance.

The results from this research show that seed viability decreases with age under accelerated ageing conditions. The present study showed that prolonged ageing results in reduced seed viability, which causes stress, promotes denaturation of proteins, and causes the death of the seed. The catabolic processes in the pretreated seeds remained somewhat suppressed, making the seeds resistant to unfavourable conditions for storage. The depletion of certain essential cellular components caused by accelerated ageing is indicated by a reduction in nucleic acid, catalase, and peroxidase activities, which are commonly utilised as accurate indicators for assessing the viability of seed. Catalase and peroxidase are also

considered possible scavenging enzymes that may effectively detoxify hazardous metabolites such as H2O2, thereby helping to relieve undesired cell-level toxicity (Bhattacharjee, 2005; Sharma, *et al*., 2012; Pati, 2019; Dumanovic, *et al*., 2021). As a result, higher catalase, protease, and superoxide dismutase activity, among other protective components, has been reported in the plant system. The findings of this study back up previous findings that rapid ageing causes a rise in protease activity as well as a decrease in anabolic enzyme activity.

Additional studies showed that storage time or the amount of time required to store seeds had a negative association with rice seed viability. The long-term aged seeds have reduced viability. The treatment of accelerated ageing results in the chemically treated seeds maintaining more vigorous seed and yielding better than the control plants. 360-day aged aromatic rice seeds reduced field performance as evidenced by decreases in plant height **(Table 3.28)**, stem circumference and inter-nodal elongation **(Table 3.29)**, yield attributes **(Table 3.27)** and biochemical parameters such as DNA **(Table 3.19)**, RNA **(Table 3.18)**, soluble **(Table 3.20)** and insoluble carbohydrate level **(Table 3.21)**, activities of catalase **(Table 3.22)**, protease **(Table 3.23)**, IAA-oxidasThe chemical induced reduction of the detrimental consequences of accelerated ageing on rice plants' overall development and metabolism suggests that pre-treatment chemicals used in plants may be retained. It is worth noting, however, that seeds that have been stored for a longer period of time may need more time to increase their percentage of germination because their rate of germination is normally slow. The data revealed that in plants raised from accelerated aged seed samples, the inception of germination to seedling emergence **(Fig. 9)**, tillering **(Fig. 13)**, elongation of stem **(Fig. 11)**, initiation of panicle to booting **(Fig. 14)**, flowering **(Fig. 16, 17 & 18)**, level of milk grain and the stage of dough grain **(Fig. 20 & 21)** were significantly delayed, with chemical pretreated samples partially alleviating the delaying action on the inception of the aforementioned developmental stages. Once more, decreased field results of plants have been linked with concomitant reductions of yield attributes, resulting in a plant's final seed yield **(Table 3.27)** impairment, produced from seeds of forced ageing. The yielding attributes, such as stem circumference diameter, internodal elongation (Table 27), seed weight, and seed number per panicle **(Table 3.29)**, The weight of 1000 seeds in pretreated seed samples has been shown to be slightly higher than in control samples. Chemically pretreated seeds have played a beneficial role in this regard, including the significant reduction of their negative effects on plant growth and yield. **(Table 3.27)**

When seeds undergoing chemical treatment were developed, plant performance and plant potential were found to be much better and greater. A comparison of the aged seeds with control seeds was used for evaluating the seed vigour. The results showed that six days of accelerated ageing is equal to a natural storage period of nine months and holds germination above the minimum certification standards for Indian seeds (Mananthi *et al*., 2015). It can be supported by the analysis that the pretreatment chemical has helped maintain seed storage capabilities and effectively reduce the harmful effects significantly. Adaptive plants' responses to environmental stresses show their high vigour and this extensive effort to modulate seed vigour by chemical processes and viability status to alleviate the specific problems and improve the metabolic status of seeds. Again, plants having higher levels of biochemical parameters in the chemical pretreated samples prove the invigorating action of the chemical. The results show that NaDK, ASA, and SADH chemical pretreated lots retained high vigour and developed more healthy seedlings, not only being more efficient in enhancing seed storage capacity but also in boosting seedling vigour than the control.

Pre-sowing seed treatment in water, mineral solutions such as $CaCl_2$, K_2SO_4, $ZnSO_4$, cobalt chloride/sulphate, KH_2O_4, $CuSO_4$, boric acid, growth regulators like manganese sulphate, ascorbic acid, kinetine, GA, benzyl adenine, and CCC, as well as products found singly or combined to fasten the process of germination; higher rate of germination and seedling vigour; enhanced resilience and crop yields. Seed treatment enhances a range of uses for seeds and seedlings to facilitate the quick and uniform germination and development of biotically, abiotically, and physiologically stressed plants. To some extent, biological agent effectiveness has improved and stabilised (Kim *et al*., 2006; Krishnaveni *et al*., 2010; Kar *et al*., 2011; Mariappan *et al*., 2013; Sharma *et al*., 2015; Dominic *et al*., 2016; Pati, 2020).

The accelerated ageing test has a positive association with field emergence and seed storage capacity. Under storage, seed health is closely associated with seed deterioration. Microorganisms, insects, pests, and fungal attacks are the major impediments to the safety of the grains, and, therefore, some precautionary measures are essential to safeguard the stored seeds. The storage fungus affects the parameters of seed quality and reduces the seed germination potential during storage. During seed storage, storage fungi affect seed quality parameters and reduce seed germination capacity. As regards the mechanism of used chemicals-induced maintenance of seed vigor, from this investigation it can be interpreted that chemicals help maintain the seed health of deteriorating seeds under adverse storage

conditions by slowing down the metabolic process and by retaining membrane integrity. According to the investigation's findings, before planting in the field, rice must be preserved for a minimum of a year or longer. Seed vigour has long been recognised as a multifaceted trait influenced by a variety of influences, including genetic background, environmental factors during seed growth, and storage stages. When it comes to storing rice seeds, temperature and environmental factors are crucial. The factors investigated thus help to understand and monitor the degradation and infection of seed during storage. Overall, there was a significant variation in the interaction impact of the processing environment, seed age, and chemical treatments. It is likely that the three pre-treating growth retardant chemicals used might have rendered seeds less susceptible to fungal damage, thereby influencing the maintenance of seed vigour for a certain period of storage.

Drought, freezing, toxic metals, salinity, UV-B radiation, and pathogen infection cause cellular homeostasis to be disrupted resulting in increased ROS production in plants. ROS is frequently produced in plants as a result of various metabolic processes in various cell compartments or as a result of eventual leakage of the chloroplasts, mitochondria, and plasma membrane electron transport processes to O_2. ROS contains free radicals, including super-oxide anion (O_2•), radicals of hydroxyl (OH) and non-radical molecules such as peroxides of hydrogen (H_2O_2), single-oxygen ($1O_2$, etc.). All ROS are highly toxic to species at high concentrations.When the amount of ROS in a cell approaches defensive mechanisms, it is stated that there is an "oxidative stress". Enhanced cell development during environmental stress can harm cells by causing lipid peroxidation, protein oxidation, nucleic acid damage, enzyme inhibition, programmed cell death (PCD) activation, and ultimately cell death (Sharma et al., 2005).

Seed degradation is linked to free radical buildup formed as a result of the process of metabolism, which is involved in ageing and plant senescence. Plant cells' free radical activity is a natural component of their metabolism, which is based on electron transmission, oxidation/reduction reactions, and molecular oxygen reactions.Free radicals, on the other hand, can cause adverse reactions in the cell and their activity is strictly controlled. The antioxidant system (AOS) in plants has a multilevel, complex network designed to fight against damaging reactive oxygen species (ROS). The ability of cells of a plant to live at high H_2O_2 concentrations is proof that they have excellent antioxidative protection mechanisms. For stressed plant survival, it is especially important for the AOS to cooperate, participate,

and improve the safety and regeneration of active reduced types of redox reactions (Shah *et al*., 2001; Sharma *et al*., 2007; Khunpon *et al*., 2018; Wang *et al*., 2019).Plants include antioxidants, detoxifying enzymes, and antioxidant compounds that scavenge ROS in order to ensure the seed longevity and removal of any free radicals "leaking" from usual metabolic processes. Free radical activity may be involved directly or indirectly in the hormonal regulation of plant senescence and seed growth and development. Abscisic acid, lipoxygenase, cytokinins, and calcium are plant hormones that have been linked to reactive oxygen species (ROS), which can attack cellular components in the absence of free radical scavengers (Rodriguez, *et al*., 1990; Sharma, *et al*., 2005; Kibinza, *et al*., 2006; Pehlivan, *et al*., 2017).

The effectiveness of chemical pre-treatment with Na-dikegulac, SADH, and ASA on maintaining storage potential and vigour of seeds can be substantiated by the comprehensive work done, and the results of this investigation are thus in conformity with the reported observations in accordance with the findings reported. The used chemicals induced regulation of free radicals and consequent improvement of seed health under storage. In several electron transfer reactions, the participation of free radicals, mostly in the form of activated O_2 species such as superoxide (O_2) or H_2O_2, is normally regulated by adequate defensive mechanisms such as the actions of superoxide dismutase, catalase, and peroxidases (Sharma *et al*., 2012). Oxidative stress can rise to higher levels, leading to cellular damage and seed degradation during seed desiccation, germination, storage, and ageing. Of the possible biophysical and biochemical causes of seed deterioration, free-radical damage leading to a disruption of the functions of the cellular membrane (Narayanan *et al*., 2016) assumes significance. On the other hand (Gondwe *et al*., 2016; Steven *et al*., 2019) found that provision of a source of free electrons significantly extended seed viability. ROS (reactive oxygen species) play a role in various stages of seed biology development. ROS are recognised as molecules that participate in cellular signalling and regulate seed development. For example, ROS has also been discovered to play a part in gene expression during early embryogenesis, dormancy, and germination, thus suggesting that quenching of naturally produced free radicals would be of advantage in controlling seed senescence (David *et al*., 2006; Barreto *et al*., 2017). In pretreated seeds under accelerated ageing conditions (Das *et al*., 2014; Pehlivan, 2017), we observed higher activity of H_2O_2-scavenger enzymes, catalase and superoxide dismutase, which are part of the seed biology growth process at different stages. Thus, in the present

study, it is quite possible that by promoting free radical enzyme activity that has made seeds tolerant of unfavourable storage, chemicals have helped to maintain essential cellular components and improve the defence mechanism.

According to the findings of this experiment, the seeds that were aged for a longer time had a decrease in viability. The viability of seeds under accelerated ageing decreases over a longer treatment period. When rice varieties were subjected to longer accelerated ageing, there was a negative association between the seed vigour and treatment time. From the present investigation, it is clear that the chemicals used at least partially alleviated the accelerated ageing-induced deleterious effects on seed germination behavior, seed metabolism, seedling growth, plant growth and metabolism at five developmental stages, as well as partially overcome them. There is little doubt that these chemicals can improve seed storage potential and maintain seed health under storage for a longer period of time, as evidenced by the current study's findings. Of the three chemicals used in this investigation, the promising effects of Na-DK on storage potential and viability extension of aromatic rice seeds in unfavourable storage conditions are obvious.

The future focus is on saving each grain that is produced, and the findings of this study will direct farmers and processors to ensure high-quality seeds on concerns about storage time and storage temperature. The extension of ageing has led to both germination and seed viability being reduced. The findings of the physiological results matched the biochemical parameters. Aging conditions have led to higher moisture content in all varieties. According to the findings, accelerated ageing had an impact on the seed quality of all rice varieties. The present study showed that the vigour of seeds from various varieties actually varies, which results in later changes in field performance. With regard to accelerated ageing, all of the research studies concluded that the variety Kalonunia is particularly susceptible, and Mohanbhog is the best variety, followed by MasinoBasmati, Musli, and Khemti varieties.

Chapter VI

Conclusion

6. CONCLUSION

Aromatic rice has a strong potential to attract rice consumers due to its flavour and deliciousness, as well as a premium price to improve the rice grower's economic situation. However, worldwide consumption has exceeded supply due to vigour losses caused by inadequate seed storage. Rice biodiversity conservation depends on the preservation of germ plasma, both in-situ and ex-situ. Seed vigour is an important factor in seed quality, as high-vigour seeds produce more uniform plants with better yields. Accelerated ageing tests allow you to assess the viability of stored seeds by exposing them to a certain temperature and relative humidity for a period of time before completing normal germination tests.

The viability of most seeds decreases with an increase in storage time, temperature, and relative humidity. There isn't much information on how long rice seeds can be stored. When compared to seedling emergence and the index of emerging speed, accelerated ageing is a good choice for a vigour test since it takes less time and produces more accurate findings. The experiments were carried out in the laboratory to investigate the influence of long-term accelerated ageing (0-, 90-, 180-, 270-, and 360-days) using five local aromatic rice types: **Kalonunia**, **MohanBhog**, **Khemti**, **MasinoBasmati**, and **Musli** of the **Darjeeling hills**, chemically processed with NaDK (1000 and 2000µg/ml), ASA (250 and 500µg/ml), and SADH (150 and 300µg/ml) or distilled water.

- From the study, it was clear that no single criteria were sufficient to be used as an index of deterioration of control seeds faster than the chemically pretreated seeds due to accelerated ageing.
- The chemical-induced arrestation of rapid loss of enzyme activity is indicative of the strengthening of the defence mechanism by the chemicals under ambient storage conditions.
- To overcome the vigour and viability status of rice seeds under ambient storage, the chemicals harden the seeds. That‟s why reduced germination behaviour and metabolic activity lead to better seed health. Thus, a conclusion can be drawn from the present investigation that the chemical NaDK can potentially enhance the seed viability of rice seeds under ambient storage.
- High relative humidity treatment increased the forced ageing process, and this reduced the levels of protein, carbohydrate, amino acids as well as enzyme activities,

which was considerably checked by seed pretreatment. However, a significant alleviation of the injurious effect was noted best in seeds that underwent pretreatment with NaDK.

- Less leakage of soluble carbohydrates and amino acids from the chemically-treated seeds used shows that NaDK, ASA, and SADH, respectively, have been tolerant to storage deterioration by preserving membrane integrity in an unfavourable environment.
- Changes in a variety of biochemical parameters examined here, which are considered reliable indices of seed vigour, help the efficacy of NaDK, ASA, and SADH, respectively, in maintaining the health of five aromatic rice seeds during the accelerated ageing time.
- The pre-treating chemical efficiently relieved the deleterious effect of forced ageing treatment. Among the chemicals used, Na-dikegulac arrested the alarming decrease in internal soluble carbohydrate levels compared to the other chemicals used, ASA and SADH, as observed in experiments.
- In this study, despite the accelerated ageing process, chemical seeds pretreated caused a mitigation of the deleterious consequences of ageing, kept the plants more vigorous than controls and influenced metabolically.
- The results demonstrate that NaDK, ASA, and SADH pretreated seed lots retained high vigour, developed more healthy seedlings, and were not just more efficient in enhancing seed storage capacity, but also more efficient in boosting seedling vigour than the control one.
- The resultant control plants from aged seeds recorded lower values for all the yield-attributing parameters compared to plants obtained from chemically pretreated seeds.
- The accelerated ageing had an impact on the seed quality of all rice varieties, which resulted in later changes in field performance. The cumulative study based on the results of all the parameters concluded that among all the experimental varieties, Kalonunia is particularly susceptible, and Mohanbhog is the best variety, followed by MasinoBasmati, Musli, and Khemti varieties.

Chapter VII

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Chapter VIII

Appendices

8. APPENDICES

APPENDIX - A

LIST OF PUBLICATIONS

1. Tamang, D. and Lama, P.C. Influence of Growth Retardants On Storage Potential of Aromatic Rice Seeds In Accelerated Ageing Condition In Darjeeling. J. of Agroecology and Natural Resource Management p-ISSN: 2394-0786, e-ISSN: 2394-0794, Vol.7, Issue 1. Jan-Mar, 2020, pp.1-4

2. Tamang, D., Lama, P.C. and Pradhan, S. Effect of Phytohormone- GA_3 in Maintainance of Viability of Highland Aromatic Rice Seeds Under Adverse Storage Condition. Int. J. of Research and Analytical Reviews. Vol. 6, Issue 2. Number 43602 (2019).

3. Lama, P.C. and Tamang, D. Effect of short term accelerated ageing and plant growth inhibitor- caumaric acid on seed vigour and viability of high land aromatic rice under adverse environmental condition of Darjeeling hill. Int. J. of basic and applied research. Vol. 9 Number 4 ISSN: 2249-3352(P) 2278-0505(E) (2019).

4. Lama, P.C. and Tamang, D. Effect of sodium-dikegulac on maintenance of viability of aromatic rice seeds under adverse storage condition. Bioscience Guardian. Vol. 6, Number 1, ISSN 2277-9497 (2016).

APPENDIX - B

LIST OF CONFERENCES/ SEMINARS AND WEBINARS ATTENDED

1. Participated in the 2nd Workshop on "Research Methodology using SPSS" during June 27th -28th, 2019, at Inspiria Knowledge Campus, Siliguri.

2. Participated in Workshop on "Green & Clean Environment" on 5th June, 2018, organized by the Department of Botany, Zoology, Microbiology & Geography Darjeeling Government College.

3. Participated on " International seminar" on 11th and 12th May 2017, organized by Department of Zoology, Darjeeling Government College, Darjeeling

4. Oral presentation on UGC sponsored national seminar "Advances in Biology: Eastern Himalayan Perspective" on 3rd and 4th Oct 2015, organized by Department of Botany and Department of Zoology, Kalimpong College, Kalimpong, Darjeeling.

5. Participated in the Himalayan Young Researchers Meet-1. Organized by G.B. Pant Institude of Himalayan Environment & Development (GBPIHED), and Indian Himalayas Climate Adaptation Programme (IHCAP), Swiss Agency for Development & Cooperation (SDC), from 7th-9th September, 2014, Kosi-Katarmal, Almora- 263-643, Uttarakhand, India

6. Participated on "National Conference on Biological and Bioinformatics of Economically Important Plants and Microbes" held from 17th -19th Feb 2012 jointly organized by Department of Botany & Bioinformatics facility, University of North Bengal, Siliguri, Darjeeling.

7. Attended the International conference entitled "Current Advances in Rice Blast Research (CARBR-2020)" organized by the Department of Biotechnology, National Institute of Technology Durgapur held during Dec. 1st – 5th , 2020.

8. Virtually attended "Prof. Asim Bothra Memorial Webinar" held on 30th September 2020, organized by Bioinformatics Facility, University of North Bengal.

9. Participated the webinar entitled "Plant Diversity In India" on 20th September, 2020, organized by Department of Botany In Collaboration with IQAC, Bagnan, Howrah, West Bengal, India.

10. Participated in online National Webinar on "Re-Imagining Domains Of Exclusions Amid Covid-19: Virtual Classroom And Deprivation Of Marginal Categories During The Pandemic" held on 19th September, 2020, organized by Department of Social Sciences &Languages in collaboration with IQAC Darjeeling Government College, Darjeeling.

11. Participated in the one-day national webinar on "Role of Indigenous Traditional Knowledge and Herbal Drugs In Combating Against Pandemic COVID-19" held on the 11th September, 2020 organized by Department of Botany and Internal Quality Assurance Cell.

APPENDIX - C

LIST OF ABBREVIATIONS

- ✓ ASA : Ascorbic Acid
- ✓ α : Alpha
- ✓ AR : Analytical reagent
- ✓ BSA : Bovine serum albumin
- ✓ C : Control
- ✓ ºC : Degree centigrade
- ✓ cm : Centimeter(s)
- ✓ dH_2O : Distilled water
- ✓ DNA : Deoxyribo nucleic acid
- ✓ ΔOD : Delta optical density
- ✓ $FeCl_3$: Ferric chloride
- ✓ fr. wt. : Fresh weight
- ✓ g : Gram(s)
- ✓ GA_3 : Gibberellic acid
- ✓ H : Hour
- ✓ HCl : Hydrochloric acid
- ✓ $HClO_4$: Perchloric acid
- ✓ $HgCl_2$: Mercuric chloride
- ✓ H_2O_2 : Hydrogen peroxide
- ✓ H_2SO_4 : Sulphuric acid
- ✓ IAA : Indole 3-acetic acid
- ✓ ISTA : International Seed Testing Association
- ✓ L. : Linnaeus
- ✓ LSD : Least Significant Difference
- ✓ M : Micron
- ✓ Mg : Microgram
- ✓ Mg : Milligram(s)
- ✓ $MgSO_4$: Magnesium sulphate
- ✓ min : Minute(s) (time unit)
- ✓ ml : Millilitre(s)

- ✓ Mm : Millimolar
- ✓ µM : Micromolar
- ✓ MH : Maleic Hydrazide
- ✓ $MnCl_2$: Manganese chloride
- ✓ MnO_2 : Manganese dioxide
- ✓ Mt : Million Tone
- ✓ (N) : Normal
- ✓ NaDK : Sodium dikegulac
- ✓ Na_2HPO_4 : Disodium monohydrogen phosphate
- ✓ NaH_2PO_4 : Monosodium dihydrogen phosphate
- ✓ NaOH : Sodium hydroxide
- ✓ NE : No Emergence
- ✓ nm : Nanometer
- ✓ Nos. : Numbers
- ✓ NS : Not Significant
- ✓ OD: Optical Density
- ✓ PGR(s) : Plant growth regulator(s)
- ✓ RH : Relative Humidity
- ✓ RNA : Ribonucleic acid
- ✓ SADH : Succnic Acid Dimethyl Hydrazide
- ✓ sec. : Second (time unit)
- ✓ t : Time
- ✓ TCA : Trichloroacetic acid
- ✓ TTC : 2, 3, 5 - Triphenyl tetrazolium chloride
- ✓ Tv : Total volume
- ✓ V : Volume
- ✓ v/v : Volume/volume
- ✓ wt : Weight
- ✓ w/v : Weight/volume

APPENDIX – D

LIST OF CHEMICALS

- Anthrone reagent (0.2%)
- Bovine Serum Albumin (0.5 mg/l)
- Calcium carbonate
- Chloroform
- 2, 4-dichlorophenol
- Diphenyl amine
- Ethanol
- Ether
- Ethyl acetate
- Ethyl alcohol
- Ferric chloride (0.5N)
- Folin-phenol reagent
- Glacial acetic acid
- Hydrochloric acid (concentrated and 0.1N)
- Hydrogen peroxide (0.0025M and 0.05M)
- Iodine
- Iodine-HCL solution
- Manganese chloride (1mM)
- Indole acitic acid (2mM)
- Methanol
- Ninhydrin solution (0.3%)
- Orcinol powder
- Orcinol reagent

- Perchloric acid (0.2M,5%,35% and 70%)
- Potassium chloride (0.05N)
- Potassium iodide
- Pyragallol (15mM)
- Salkowski reagent
- Sodium hydroxide (0.5N)
- Sodium carbonate
- Starch solution (0.1%)
- Sulphuric acid (concentrated, 5%, 10% and 25%)
- Titanium sulphate (0.1%)
- Trichloroacetic acid (10% and 50%)
- Yeast RNA
- 300 μM sodium phosphate buffer (pH 6.5)
- 0.05 M sodium phosphate buffer (pH 6.5)
- 0.1M sodium phosphate buffer (pH 5.7, 6.4 and 6.8)
- 0.2M sodium phosphate buffer (pH 6.1)
- 0.1M sodium acetate buffer (pH 4.0 and 5.0)
- 0.5M sodium citrate buffer (pH 6.5)

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Influence of Plant Growth Retardants on Storage Potential of Aromatic Rice Seeds in Accelerated ageing Condition in Darjeeling

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Abstract—*The present study was carried out in five aromatic rice varieties of Darjeeling hill viz. KaloNunia, Mohan Bhog, Khemti, Masi no Basmati and Musli for analyzing leaching of soluble carbohydrate and free amino acids during seed deterioration by subjected them to long term accelerated ageing treatment (95% relative humidity, RH) for 365 days (0, 90, 180, 270 and 360 days). Prolongation of ageing led to deterioration of both germinability and seed viability and increased leaching of soluble carbohydrate and free amino acids. These changes are directly proportional to seed germination and metabolism. However, some diminution of leaching was noted in the chemically pretreated seed lots with Ascorbic acid (ASA) with concentration of 250 and 500µg/ml, Succinic acid 2,2-dimethylhydrazide (SADH) with 150 and 300µg/ml, Sodium dikegulek (NaDK) with 1000 and 2000µg/ml compare to distilled water (Control) before accelerating ageing. The study concludes that accelerated ageing showed effect on seed quality of all the varieties of rice. All the test experiments performed concluded that the deleterious effect of seed leaching of soluble carbohydrate and free amino acid was substantially alleviated and ameliorated best by NaDK then followed by SADH and ASA respectively.*

Keywords: *Aromatic rice; Accelerated ageing; Ascorbic acid; Succinic acid 2,2-dimethylhydrazide Sodium dikegulac.*

INTRODUCTION

Rice aroma is the most striking characteristics of high quality rice and has gained importance as a quality character. The demand of aromatic rice is increasing in both domestic and international markets (Sakthivel K *et. al*., 2009). Rice is the most important food crop and life for thousands of millions of people. By the year 2025, about 760 million tons of paddy needs to be produced to meet the increasing demand due to increase in world population, this requirement is 35 percent more than actual rice production (Duwayri *et. al*., 2000).

A good quality rice seeds are often free from diseases and possess high germinability and vigor but obtaining high vigour seeds needs scientific managements and proper techniques for harvesting, processing, treatments and storage (Chhetri S, 2009). Seed vigour influences the productivity, quality and also the storability of seeds. Seed storage may influence seed viability and may reduce seed vigour, depending on the storage conditions and the length of storage period. The two most important factors that influence the life span of seeds are relative humidity and temperature and their effects are highly interdependent (Copeland and McDonald, 1995). Determination of seed storability and vigour in rice seeds seems to be more important recently and one of the most important is accelerated aging test which is a physiological stress test. The basis for this test is that higher vigour seeds tolerate the high temperature-high humidity treatment and thus retain their capability to produce normal seedlings.

Seed treatment is one of the important quality aspects of seeds. Treated seeds are protected from pests that attack seeds and seedlings and can improve seed quality and increase yields also (Powell, 2006). The possibility of prolonging the vigour and viability of stored seeds by chemical manipulative technique has been explored by a number of workers. To maintain seed viability, various methods have been suggested along the time (Aschermann-Koch *et. al*.,1992, Bhattacharjee *et.al*., 1984, 1993,1995, Rai A,.1999, Draganic and Lekic, 2012, Lama *et.al*., 2016)

Maintenance of vigour and viability of seeds is an important problem in agriculture and horticulture. The two environmental factors, i.e. temperature and relative humidity (RH), have profound influence on seed health under storage (Copeland and McDonald, 1995: Desai *et.al.*, 1997). In recent years, some effective physical and chemical manipulative techniques have been developed by seed technologists to get rid of such climatic as well as biotic hazards which conducive to earlier deterioration of

stored seeds . There are some reports that hydration-dehydration treatment as well as treatment of seeds with chemicals of diverse nature (salts, phenols, organic acids, essential oils, plants growth regulators, bio products) can favourably influence the viability status of seeds (Chhetri *et.al.*, 1993; Bhattacharjee *et.al.,*1984, 1986, 1993, 1999, 2012 & Sasikala K *et.al*., 2018).

Seed deterioration is a major problem in agricultural production. The climatic condition of Darjeeling hill is more acute due to high humidity. Keeping this problem in mind, an attempt was made to enhance the storage potential of rice seeds which undergo forced deterioration under adverse storage environment/ accelerated ageing. Thus the major, objective of this work was to test the efficacy of growth inhibitors ASA, SADH and NaDK on the alleviation of seed deterioration under storage.

MATERIALS AND METHODS

Aromatic rice Seed lots of 5 varieties KaloNunia, MohanBhog, Khemti, MasinoBasmati and Musli were collected from local farmers of Darjeeling, West Bengal, India. After collection, the seed lots were separated from husk and healthy, undamaged seeds were selected for experimental purposes. During the experimental period the environmental conditions of Darjeeling were as follows: Temperature: 6-22 degree Celsius, Relative humidity: 90-95+_ 2%.

This experiment was performed under artificially imposed environmental conditions called accelerated ageing for obtaining a relatively uniform and expeditious result. In case of long term accelerated ageing condition, starting from 0, 90, 180, 270 and 360-days, analyses were made at 90-days intervals up to 365days (1 year) after imposing ageing conditions and then experiments was terminated. After the surface sterilization with 0.1% mercuric chloride ($Hgcl_2$) for 90sec., all the 5 varieties of aromatic rice seeds KaloNunia, MohanBhog, Khemti, Masino Basmati and Musli were separately pre-soaked with aqueous solutions of Ascorbic acid (ASA) with concentration of 250 and 500µg/ml, Succinic acid 2, 2-dimethylhydrazide (SADH) with 150 and 300µg/ml, Sodium dikegulek (NaDK) with 1000 and 2000µg/ml or distilled water for 6 hours and then dried back to original weight of seeds. After 48 hours intervals such soaking dry treatments were repeated 3 times to make the total duration of pre-treatment of 18 hours. This mode of pre-treatment enabled maximum pre-treatment of the chemical while avoiding the commencement of germination. After complete pre-treatment of seed lots, the pre-treated seed lots (200g) each were put into separate cloth bags and thus stored in a desiccators in which an environment of 98.2% relative humidity was pre imposed by keeping 250 ml 5.96% sulphuric acid (vol/vol) within it. This experimental setup was kept allowing the seeds to experience forced ageing treatment and Sulphuric acid was changed periodically to restore the desired relative humidity within the dissector throughout the experimental period.

Free amino acid levels from the seed leachate of each treatment and each ageing period (0, 90, 180, 270, 360 days) were analysed after immersing 1g seed sample in distilled water for 16h. From the leachate stock, free amino acid level was quantified following the method of Moore and Stein (1948) modified by Bhattacharjee (1984). Sampling procedure of soluble carbohydrates from seed leachates was the same as done in case of leachable amino acids, and from the same leachate stock, soluble carbohydrate level was determined following the method of McCready *et.al.* (1950) with slight modification.

RESULTS AND DISCUSSION

In the long term accelerated ageing period, the high RH (relative humidity) treatment rapidly enhanced the rate of ageing, senescence of seeds and viability status was found to be low. Leaching of soluble carbohydrate and amino acids from the seed samples KaloNunia, Krishna Bhog, Khemti, Masino Basmati and Musli increased with advancement of ageing duration irrespective of treatment when data were recorded in 0, 90, 180, 270 and 360 days respectively. However, this increase was significantly reduced most in NaDK, then in SADH and lastly in ASA pretreated seed samples, regardless of the concentrations used, magnitude of the decline was less in all NaDK pre treated seed lots. The results are indicative of the fact that accelerated ageing caused to damage seed membrane which consequently resulted in the higher leakage of soluble carbohydrate and free amino acid in all control samples but in pre treating chemicals alleviated this deleterious effect to a considerable extent.

Leaching of soluble carbohydrate (Figure-2) from the accelerated ageing storage seeds showed positive result with the period of accelerated ageing. Data showed that the 0, 90, 180, 270 and 360 days respectively after ageing significantly increased leaching but during subsequent analyses the pre treating chemicals, regardless of its concentration, checked the higher leakage of sugars in all seed sample. Accumulation of amino acids in seed leachates (Figure-1) went on increasing in control seed samples of all the crop seeds with advancement of ageing duration. The same trend was apparent in case of chemical-pre treated seed samples but there the rate of increase was considerably slowed down at all concentrations. The higher concentration of NaDK was found effective in enhancement of enzyme activity in accelerated ageing duration.

Leakage of soluble carbohydrate and amino acids from the seeds is indicative of possible damage to seed membranes. There are numerous reports that the seed membranes are affected in deteriorating seeds leading to a progressive loss of viability. Biochemical parameters are increasingly used as indicators of seed vigour and viability. In this study, NaDK substantially alleviated the loss of sugars and amino acids followed by SADH and ASA respectively and hence the role for these chemicals in retaining membrane integrity and thereby decreasing loss of seed viability is the subject of much debate. Solute leaching was

associated with reduced vigour and germ inability activity of artificially aged seeds. A beneficial effect of NaDK on the maintenance of seed viability can also be postulated from these parameters, as the chemical was found to retard partially the rapid loss of vigour and enzyme activity.

Data showed that in long term accelerated ageing level of sugars and enzyme gradually declined in control samples with ageing duration and this declining trend was considerably slowed down by the pre treating chemicals. The above results, therefore, p oint out that although deterioration is a common phenomenon both in treated and in control seed samples, the leaching processes within the treated seeds remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environments. That the pre treating chemicals are efficient in substantial alleviation of the damaging effect of accelerated ageing can be supported from the analyses. Data showed that progressively declined in long-term ageing experiment is generally used as a reliable index for the evaluation of seed viability.

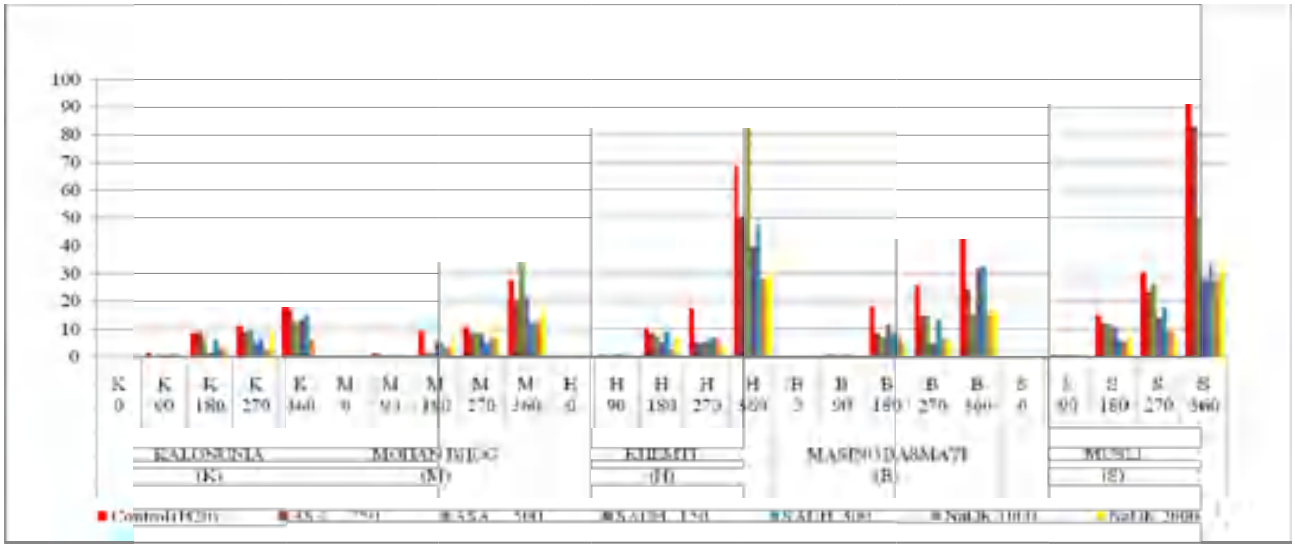

Figure 1: Effect of long term accelerated ageing and seed pretreatment with aqueous solutions of Ascorbic acid (ASA) concentration of 250 and 500µg/ml, Succinic acid 2, 2-dimethylhydrazide (SADH) 150 and 300µg/ml, Sodium dikegulek (NaDK) 1000 and 2000µg/ml or distilled water (control) on leaching of free amino acids (mg/g fresh weight) contents in seeds of aromatic rice varieties : Kalo Nunia, Mohan Bhog, Khemti, Masino Basmati and Musli. Mature and healthy seeds were pretreated with the chemicals and distilled water for 18 hours. Data were recorded at an interval of 0-, 90-,180-, 270- and 360- days respectively.

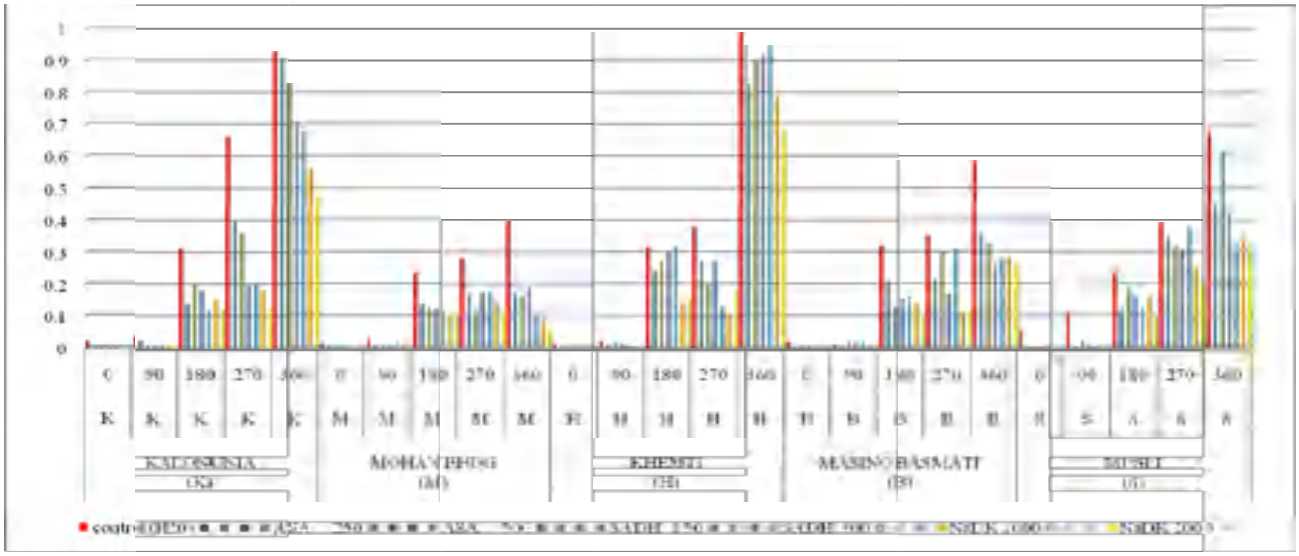

Figure 2: Effect of long term accelerated ageing and seed pretreatment with aqueous solutions of Ascorbic acid (ASA) concentration of 250 and 500µg/ml, Succinic acid 2, 2-dimethylhydrazide (SADH) 150 and 300µg/ml, Sodium dikegulek (NaDK) 1000 and 2000µg/ml or distilled water (control) on leaching of soluble carbohydrate (mg/g fresh weight) contents in seeds of aromatic rice varieties : KaloNunia, MohanBhog, Khemti, MasinoBasmati and Musli. Mature and healthy seeds were pretreated with the chemicals and distilled water for 18 hours. Data were recorded at an interval of 0-, 90-,180-, 270- and 360- days respectively.

Thus from a number of viability indices it can be concluded that NaDK may be used as abest seed potentiating (or hardening) agent among three chemicals used in experiment. Further work is in progress to establish its role in enhancing the storage potential of seeds.

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Effect of short term accelerated ageing and plant growth inhibitor -coumaric acid on seed vigiour and viability of high land aromatic rice under adverse environmental condition of Darjeeling hill

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Abstract

Short-term accelerated ageing treatment (98.2% relative humidity, RH) on two highland varieties of aromatic rice seeds (Dudhraj and Birimful) reduced the germinability of seeds and deteriorated seed vigour and viability. In short term accelerated ageing period for 30-days seed membrane was damaged significantly and increased leaching of carbohydrate, free amino acids and protein, soluble and insoluble carbohydrate contents in seed kernels. The total dehydrogenase, catalase activities were lesser with ageing period. These all changes in metabolism are directly proportional to seed germination and metabolism. Pretreatment of the seeds with Coumaric acid (100, 250, 500 µg/ml) for 24 hours before accelerating ageing conditions substantially alleviated all the deleterious effects of ageing.

Keywords: Aromatic rice, Effect of Phytohormone-coumaric acid, Post harvest, Pretreatment, Seedvigour and viability.

Introduction

Maintenance of viability, vigour and storing of seeds is a problem in tropical region where high temperature and high relative humidity accelerate the seed deteriorations which ultimately resulting non-viability (Dey et al., 2012). Seed deterioration is a major problem in agricultural and horticultural production. This is a natural catabolic process which terminates seeds life span resulting in complete loss of viability. Furthermore, the pathogenic invasion as well as the adverse environmental conditions enhanced the process. This inevitable natural detrimental process particularly pathogen and adverse environment –induced accelerated ageing leading to rapid deterioration of seeds is a matter of concern to the seed technologists, crop growers and seeds men

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associated with seed industry. Storing of seeds is a serious problem in tropical and subtropical countries like India where high temperature and high relative humidity greatly accelerate seed ageing, deterioration and non viability. The problem of retention of seed vigour in Darjeeling and surrounding areas is much more acute because of the extremely high relative humidity which is conducive to the growth of micro-organisms. As most crop seeds require storage for either one or several planting seasons, agriculturists and horticulturists of this region are often handicapped with respect to maintenance of standard seed vigour under ambient storage environment (Bhattacharjee et al 1993, Rai A.,1999).

Rice, one of the major stable food grains throughout the world requires storage for one or more planting seasons before cultivation. One of the sensory attributes of rice preferred by consumers is its aroma. Aromatic rice has become popular and continues to command a higher price than ordinary non-aromatic rice both in the local and international markets. Aromatic rices have been preferred over non-aromatic rice for over hundreds of years. They have premium value owing to their unique aroma and quality. Aroma is one of the most important characteristics of rice, especially when taking consumers acceptance as a criterion. The demand of aromatic rice is increasing in both domestic and international market (Sakthivel K et al., 2009)

For a long time researchers are involved themselves to develop more suitable techniques to keep the seed health well during storage. More recently infusion of fungicides, growth regulators, pesticides, bio-products, bio-ingredients, agro-chemicals and herbicides into the seeds prior to germination is reported to alleviate the impact of adverse factors on seed quality and performance (Janmohammadi *et al*, 2008). Seeds invigouration implies an improvement in seed performance by any post harvest treatment such as soaking-drying with chemicals, growth regulators etc. resulting an improvement in germination percentage, greater storability, high yielding capability and high vigour of plants than the corresponding untreated seeds (Basu, 1990). To maintain seed viability, various methods have been suggested along the time (Aschermann-Koch *et al.*, 1992., Basu and Dey, 1983., Berherea and Roul, 1995., Bhati and Rathore, 1987., Prasad *et al.*, 2005., Burries, 2002., Deosarkar *et al.*, 2002).

The post-harvest system must be improved, including infrastructure development and also the dissemination of technologies, allowing small and medium farmers to prevent food losses. The rice post-harvest system requires improvement in the use of resources for research and development, particularly with regard to the level of post-harvest losses. These losses are attributed to a combination of factors during production and post-production operations (De Padua, 1999). In India, paddy occupies the first place both in area under cultivation and grain production (Babu et al., 2014). The postharvest system for rice deserves special attention rice is a major staple food in the world and is mostly produced in developing countries where the implementation of postharvest technologies is urgent in order to prevent food rice losses. It has been estimated that rice postharvest losses may be as high as 16 percent due to use of old and outdated methods of drying and milling, improper and unscientific methods of storage, transport and handling (Mejhia 2015).

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It has been estimated that total post-harvest losses of paddy at producers level was about 2.71% of total production. To minimize post-harvest losses, precautions should be taken to follow proper post-harvest practices. They include timely harvest, use of proper method of harvesting; avoid excessive drying, fast drying and rewetting of grains. To avoid storage losses maintaining optimum moisture content i.e. 12% for longer period and 14% for shorter storage period is essential (Pandey P.H. 1998). Physical and chemical manipulative methods are adopted by the physiologists to prolong the post harvest seed storage capacity maintaining the seed vigour and viability. There are several classes of chemicals as manipulative agents viz. hormones, retardants, redox chemicals, phenols, vitamins and salts which established a significant efficacy on maintenance of seed health under storage Coolbear, Francis and Grierson, 1984, Bhattacharjee et. al 1984, 1993, Rai 1999, , Draganic and Lekic, 2012, Ojha 2013, Lama et.al 2016, Sasikala K et al., 2018)

It has been documented in different literatures that there were numerous varieties of aromatic rice available in Himalayan region. But, due to wrong methods of collection, unscientific post harvest storage system and introduction of high Yield variety, the indigenous varieties started to loss their existence in their own natural habitat. It is recorded that more than twenty-one different aromatic rice varieties are available in the three sub divisions of Darjeeling district in the high land areas. All these varieties are cultivating by local farmers in their own traditional system. The gradual depletion oftheir cultivation practices and damage during the post harvest period due to environmental and others pathogenic factors, aromatic rice varieties needs an urgent scientific interference.

Seed deterioration is a major problem in agricultural production. The climatic condition of India greatly accelerates the seed ageing phenomenon under the ambient storage condition. The problem in high hill of Darjeeling is more acute due to high humidity. Keeping this problem in mind, an attempt was made to enhance the storage potential of rice seeds which undergo normal deterioration under adverse storage environment. Thus the major, objective of this work was to test the efficacy of growth regulators on the alleviation of seed deterioration under storage.

Materials and methods

Aromatic rice Seed lots of 2 varieties (Dudhraj and Birimful) were collected from local farmers of Darjeeling, West Bengal, India. After collection, the seed lots were separated from husk and healthy, undamaged seeds were used for experimental purposes.

During the experimental period the environmental conditions of Darjeeling were as follows: Temperature: 20-22 degree Celsius, Relative humidity: 85+_ 2%.

Experimental condition and seed pre-treatment

This experiment was performed under artificially imposed environmental conditions called accelerated ageing for obtaining a relatively uniform and expeditious result. In case of short term accelerated ageing condition, starting from 0-day, analyses were made at 10-day intervals up to 3 weeks after imposing ageing conditions and then experiments was terminated.

After the surface sterilization with 0.1% mercuric chloride ($Hgcl_2$) for 90sec., all the seed varieties were separately pre-soaked with aqueous solutions of Coumaric acid with concentration of

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100, 250 and 500µg/ml or distilled water for 18 hours and then dried back to original weight of seeds. After 8 hours intervals such soaking dry treatments were repeated 3 times to make the total duration of pre-treatment of 24 hours.

This mode of pre-treatment enabled maximum pre-treatment of the chemical while avoiding the commencement of germination. After complete pre-treatment of seed lots, the pre-treated seed lots (20g) each were put into separate cloth bags and thus stored in a desiccators in which an environment of 98.2% relative humidity was pre imposed by keeping 250 ml 5.96% sulphuric acid (vol/vol) within it. This experimental setup was kept allowing the seeds to experience forced ageing treatment and sulphuric acid was changed periodically to restore the desired relative humidity within the dissector throughout the experimental period.

Biochemical analysis

The soluble and insoluble carbohydrate levels from the seed kernel were analysed, following the method of Mc Cready et.al.(1950). The activity of total dehydrogenase of intact seeds was anslysed by the reaction of TTC according to the method of Rudrapal and Basu (1979). Protein and free amino acids levels of seed kernels were analysed by following the methods described by Lowery et.al. (1951); Moore and Steinn (1948). The seed enzyme activity, particularly, alpha amylase was extracted and estimated from the pre-treated seed lots following the method described by Biswas and Chouddhuri (1978). All the data were statistically analysed at the treatment and replication levels by Panse and Sukhatma (1967).

Results and discussion

Maintenance of vigour and viability of seeds is an important problem in agriculture and horticulture. The two environmental factors, i.e. temperature and relative humidity (RH), have profound influence on seed health under storage (Copeland and McDonald, 1995: Desai et.al, 1995). In recent years, some effective physical and chemical manipulative techniques have been developed by seed technologists to get rid of such climatic as well as biotic hazards which conducive to earlier deterioration of stored seeds .In the literature dealing with seed viability, there are some reports that hydration-dehydration treatment as well as treatment of seeds with chemicals of diverse nature (salts, phenols, organic acids, essential oils, plants growth regulators, bio products) can favourably influence the viability status of seeds (Basu et.al. 1979; Savino et.al,1979; Pathak and Basu,1980; Chhetri et.al.1993; Bhattacharjee et.al.,1984,1986,1993, 1999, 2013 & Sasikala K et al., 2018).).

In the short term accelerated ageing period, the high RH (relative humidity) treatment rapidly enhanced the rate of ageing and senescence of seeds and viability status was found to be appreciably low. The results are indicative of the fact that accelerated ageing caused to damage seed membrane which consequently resulted in the higher leakage of soluble carbohydrate and pre treating chemical alleviated this deleterious effect to a considerable extent. Leaching of soluble carbohydrate (Figure- 1) and free amino acids (Figure-2) from the accelerated ageing storage seeds showed positive with the period of accelerated ageing. Data showed that the 0-day after ageing coumaric acid at its highest concentration significantly increased leaching but during subsequent analyses the pre treating chemical, regardless of its concentration, checked the higher leakage of sugars in all seeds.

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Accumulation of amino acids in seed leachates went on increasing in water soaked seed samples ofall the crop seeds with advancement of ageing duration. The same trend was apparent in case of chemical-pre treated seed samples but there the rate of increase was considerably slowed down at all concentrations. Unlike the changes of leakage products, protein contents (Figure-3) of seed kernels gradually declined keeping pace with days of accelerated ageing both in water-soaked and chemical pre treated seed samples. But the pre treating chemical helped to maintain the protein level to a considerable extent at later observation periods. In case of soluble and insoluble carbohydrate contents (Figure-7 & 8), with the progress of ageing, sugar levels in the kernels increased upto 10 days and followed by gradual decrease in level contents with ageing duration. There were a significant increase of insoluble sugar level was recorded in the later ageing. All concentrations of Coumaric acid were found almost equally efficient in averting the loss of sugar content to a considerable extent. So far the overall changes of insoluble carbohydrate level is concerned, a clear reverse picture was found noted with the changes of soluble carbohydrate level. There was a drastic fall of TTC stainability in seed kernels when they experienced prolonged accelerated ageing for 30 days (Figure-4). In the chemical pre treated seed samples parallel reduction of TTC stainability was found but comparatively higher percentage of TTC-stained seeds was recorded than in the control samples. Similarly, total dehydrogenase and catalase activities of seed samples (Figure- 5 & 6) were observed that the extent of massive fall of activity was relieved in GA pre treated samples. The higher concentration of Coumaric acid was found effective in enhancement of enzyme activity in accelerated ageing duration.

During accelerated ageing period, changes of leachable carbohydrates and amino acids were associated with proportional shift metabolism within seed kernels of the seeds. Efficacy of Coumaric acid on the maintenance of seed health can also be supported from the changes of a number of biochemical parameters analysed here which are considered as reliable indices of seed vigiour. Data showed that in short term accelerated ageing level of protein and sugars gradually declined in control samples with ageing duration and this declining trend was considerably slowed down by the pre treating chemical.

The above results, therefore, point out that although deterioration is a common phenomenon both in treated and in control seed samples, the catabolic processes within the treated seeds remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environments. That the pre treating chemical is efficient in substantial alleviation of the damaging effect of accelerated ageing can be supported from the analyses of total dehydrogenase activity of seed kernels as well as from TTC-stained seeds. Data showed that both dehydrogenase activity and percent TTC-stained seeds progressively declined in short-term ageing experiment. Dehydrogenase activity is generally used as a reliable index for the evaluation of seed viability (Copeland and McDonald., 1995). There are also reports that as seeds age, they lose vigour which is evaluated by counting percentage TTC-stained seeds, depending on their viability status (Halder, 1981).

In this investigation, the observed chemical induced alleviation of the rapid loss of metabolic activities under accelerated ageing condition, are indicative of the fact that Coumaric acid helped the seeds to tolerate the unfavourable storage environment. There are numerous reports that seed

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membranes are highly affected in deteriorating seeds, resulting in increased permeability and decreased germination of seeds. Thus, it seems possible that the chemicals used in this investigation have some potential for retaining membrane integrity at least for certain duration.

Figure-1.

Effect of short term accelerated ageing and seed pretreatment with Coumaric acid (Cou 100, 150 and 500 µg/ml) on leaching of soluble carbohydrate (Sol., mg/g fresh weight) contents in seeds of aromatic rice varieties :Dudhraj (DJ) and Birimful (BF).Mature and healthy seeds werepretreated with the chemicals and distilled water for 24 hours. Data were recorded from seed leachate after immersing each sample in 20 ml deionised distilled water for 16 hour.

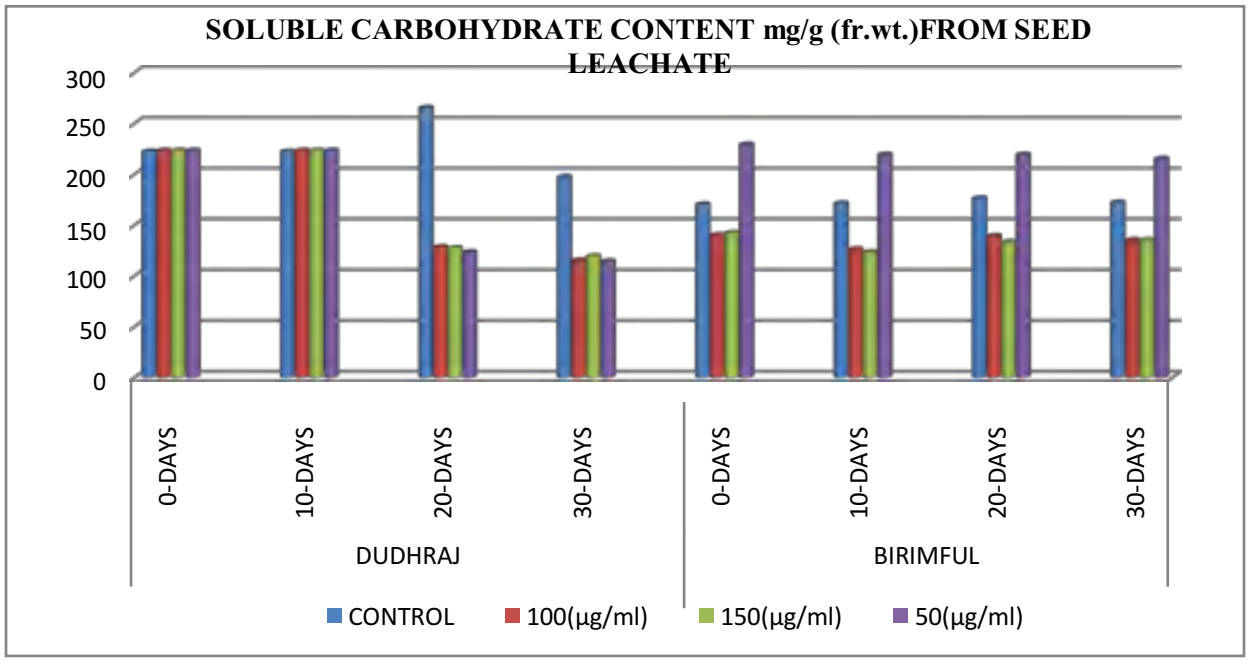

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Figure-2.

Effect of short term accelerated ageing and seed pretreatment with Coumaric acid (Cou 100, 150 and 500 µg/ml) on leaching of free amino acids (FAA., mg/g fresh weight) contents in seeds of aromatic rice varieties : Dudtraj (DJ) and Birimful (BF). Mature and healthy seeds were pretreated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30- days respectively.

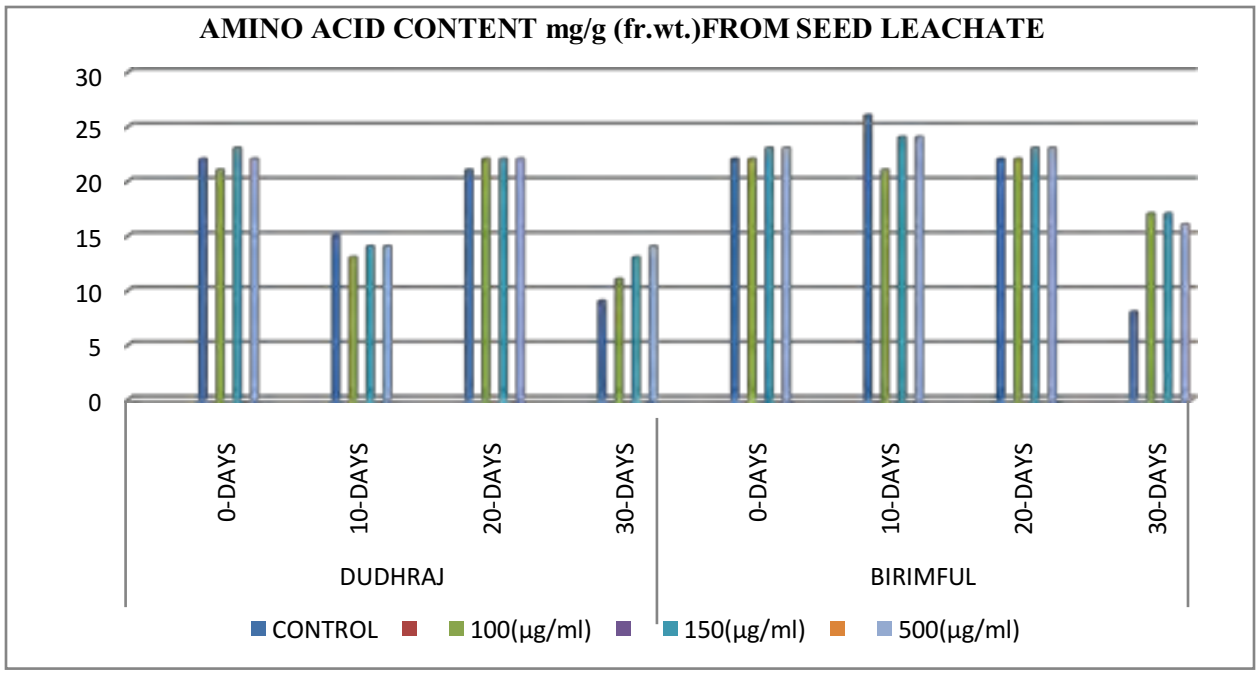

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Figure-3.

Effect of short term accelerated ageing and seed pretreatment with Coumaric acid (Cou 100, 150 and 500 µg/ml) on changes of protein (Pro., mg/g fresh weight) contents in seeds of aromatic rice varieties: Dudhraj (DJ) and Birimful (BF). Mature and healthy seeds were pretreated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30- days respectively.

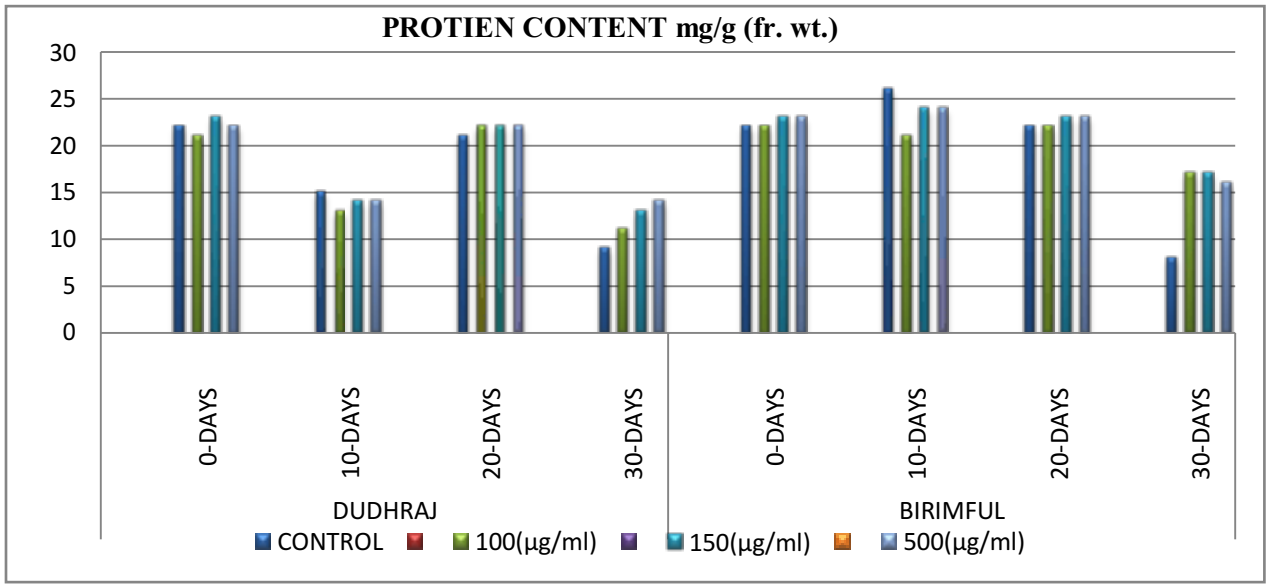

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Table-4.

Effect of short term accelerated ageing and seed pretreatment with Coumaric acid (Cou 100, 150 and 500 µg/ml) on percentage TTC-stained seeds of aromatic rice varieties: Dudhtraj (DJ) and Birimful (BF). Mature and healthy seeds were pretreated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30- days respectively.

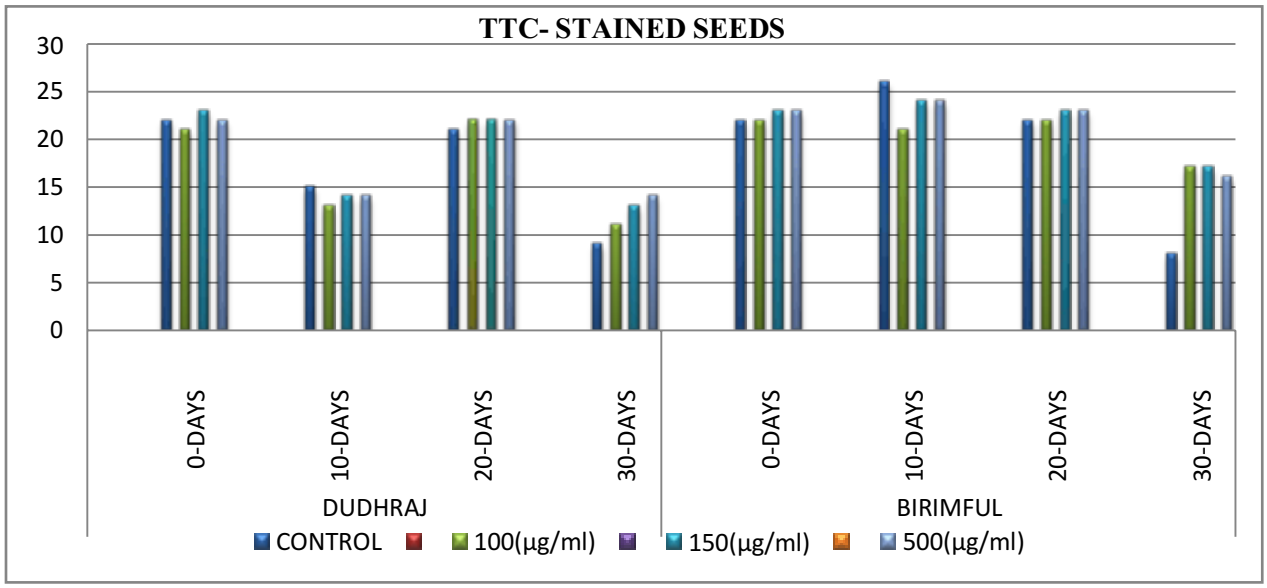

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Figure-5.

Effect of short term accelerated ageing and seed pretreatment with Coumaric acid (Cou,100, 150 and 500 µg/ml) on changes of dehydrogenase activity (Dehyd., ΔOD /g/ml) in seeds of aromatic rice varieties: Dudhraj (DJ) and Birimful (BF). Mature and healthy seeds were pretreated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30- days respectively.

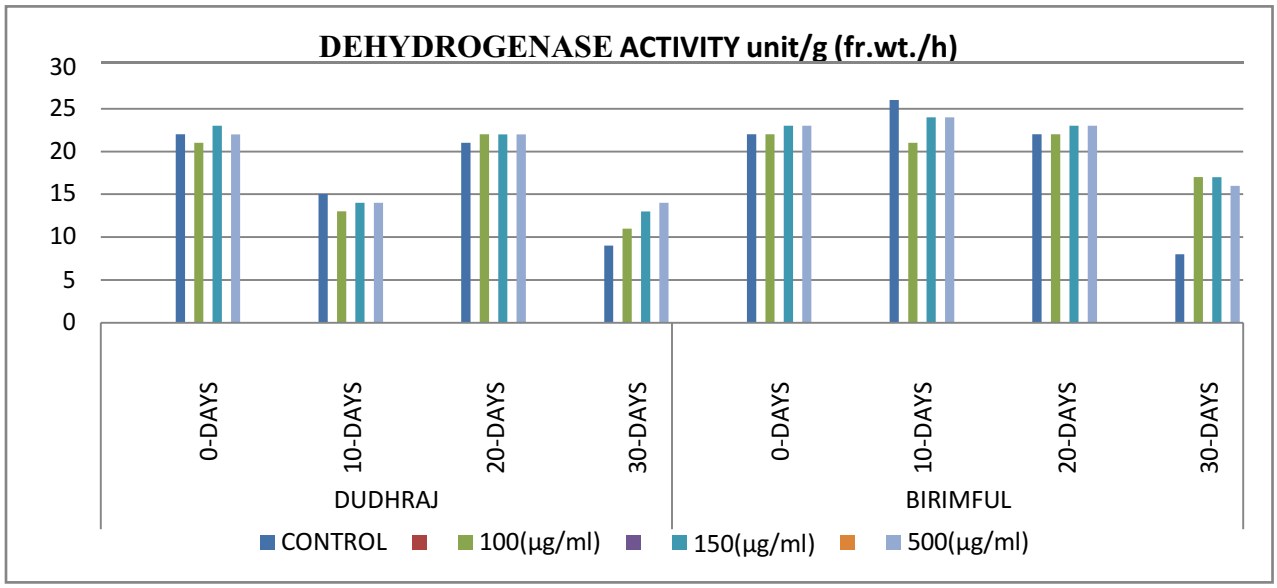

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Figure-6.

Effect of short term accelerated ageing and seed pretreatment with Coumaric acid (Cou,100, 150 and 500 µg/ml) on changes of catalase activity [Cat., (ΔOD x Tv)/ (t xv)] in seeds of aromatic rice varieties: Dutraj (DJ) and Brimful (BF). Mature and healthy seeds were pretreated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30- days respectively.

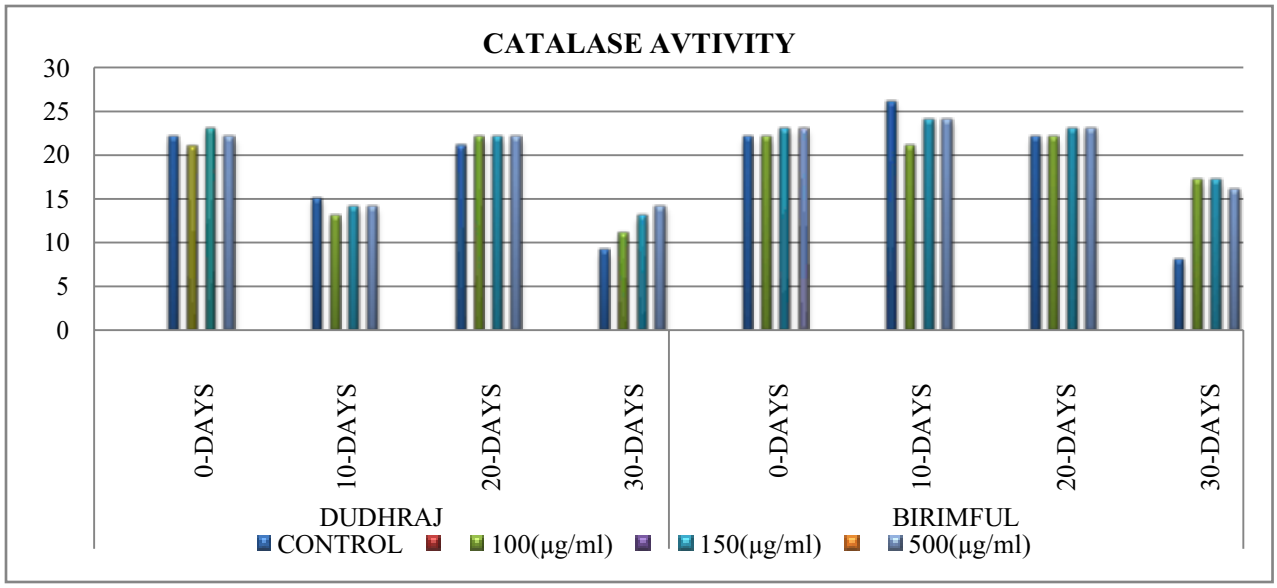

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Figure-7.

Effect of short term accelerated ageing and seed pretreatment with Coumaric acid (Cou,100, 150 and 500 µg/ml) on changes of soluble carbohydrate(Sol., mg/g fresh weight) contents in seeds of aromatic rice varieties : Dudhraj (DJ) and Birimful (BF). Mature and healthy seeds were pretreated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30- days respectively.

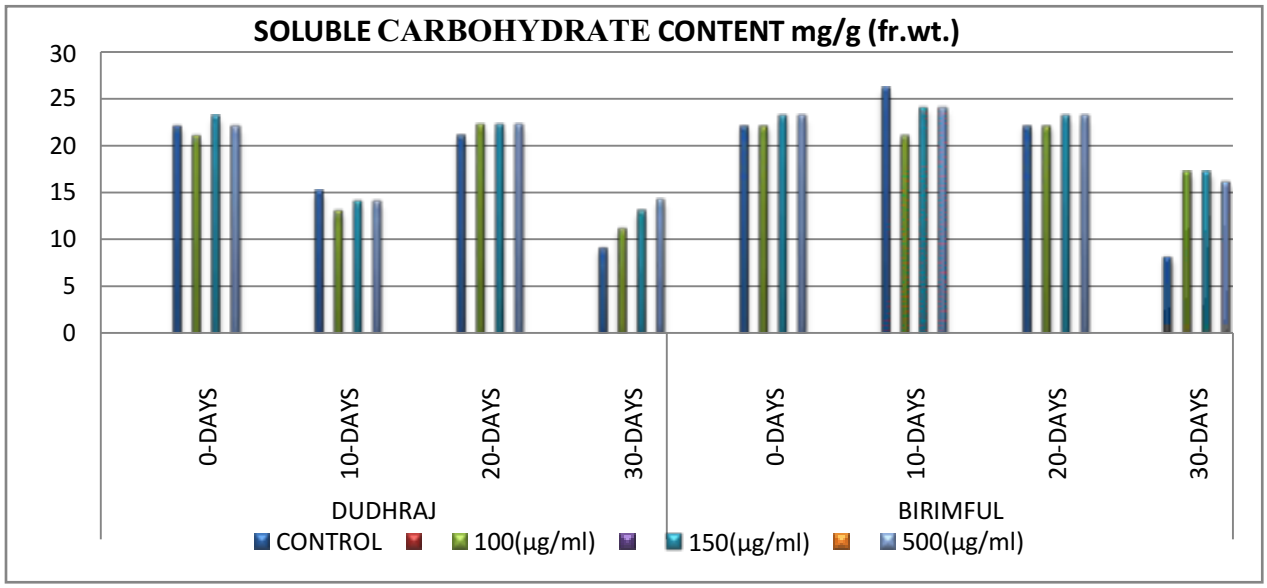

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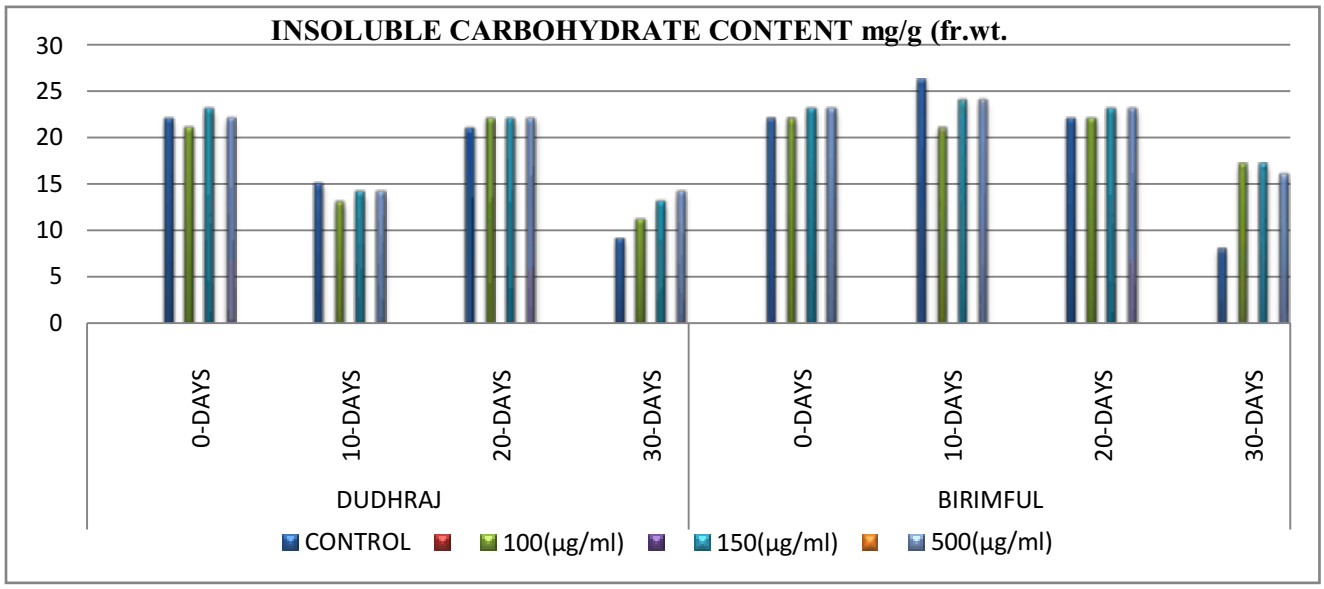
Figure-8.

Effect of short term accelerated ageing and seed pretreatment with Coumaric acid (Cou,100, 150 and 500 µg/ml) on changes of Insoluble carbohydrate (Insol., mg/g fresh weight) contents in seeds of aromatic rice varieties: Dutraj (DJ) and Brimful (BF). Mature and healthy seeds were pretreated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30- days respectively.



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EFFECT OF PHYTOHORMONE- GA_3 ON MAINTENANCE OF VIABILITY OF HIGHLAND AROMATIC RICE SEEDS UNDER ADVERSE STORAGE CONDITION.

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Abstract: **Biochemical activities of the seed metabolism were analyzed after seed pre-treatment and accelerated ageing on four varieties of highland aromatic rice (Krishnabhog, Musli, Kattaka and Dhanasae) from Darjeeling hill. Short term accelerated ageing treatment (98.2% relative humidity, RH) on four highland aromatic rice for 30-days, damaged seed membrane significantly and increased leaching of carbohydrate, free amino acids, protein, soluble and insoluble carbohydrate contents in seed kernels. The leaching of sugar, amino acids increased with progress of ageing duration. Such changes were associated with proportional reduction in the level of protein, insoluble carbohydrate and the total activity of dehydrogenase and α-amylase activities. Rice seeds pre-treated with growth hormone GA_3 (50, 150 and 300µg/ml) for 24 hours prior to accelerated ageing showed damaged seed membrane and increased the leaching of soluble substances and declining the levels of vital cellular components such as carbohydrate, protein, total dehydrogenase, alpha amylase enzyme. Hence, GA_3 growth promoter hormone was ineffectual to arrest the leaching of cellular components and seed kernels deterioration under accelerated ageing treatment.**

Keywords: **Aromatic rice, Phytohormone-GA_3., Accelerated ageing, Seed pre-treatment**

1. Introduction

India contributes about one-third of the world acreage under rice cultivation. Rice, *Oryza sativa* L. the staple food of nearly one-half of world's population, contributes over 20% of the total calorie intake of man. India is one of the worlds, second only to China, cultivating 43 million hectares annually, which is about a third of the World acreage under rice. Rice is the major cereal crop of India, being available in over 5000 varieties differing with respect to size, texture glutinous nature, aroma and cooking quality. Among all the Asian countries, India is a prominent rice growing country accounting for about 20% of rice production (Babu et al.,2014)

In the Indian subcontinent, aromatic rices are categorized as Basmati and non-Basmati. Basmati rice- the scented pearl is nature's gift (Singhal et al., 2002). The Basmati types are characterized by long, slender grains having high kernel elongation after cooking. The non-Basmati aromatic rices also have one or more of the Basmati characteristics, but not all of them. Especially, they have small and medium kernel length (Singh et al, 2000). Aromatic rice occupies a prime position and is traditionally grown in the Himalayan foothill regions of India. Aromatic rices have been preferred over non-aromatic rice for over hundreds of years. Grain aroma is one of the most striking characteristics of high-quality rice, especially when taking consumer acceptance as acriterion (Vanavichit et al., 2010).

Phytohormones are chemical substances and when applied in small amounts, bring changes in the phenotype and growth of the plant either by enhancing or by stimulating the natural growth regulatory system. Growth regulators can improve the physiological efficiency and can enhance effective portioning of accumulates from source and sink in the field crops (Lee et al., 1999, Khan et al., 2007, Kazaz et al., 2010, Lama et al., 2014). There are many reports which indicate tha t application of growth inhibitors defers scenescence and prolongs storage life of seeds by delaying deterioration process and can alter the levels of some macromolecules of seeds (Bhattcherjee et al., 1998, Rai et. al., 1999 & 2000, Ojha et al., 2012,

Lama et al., 2016). Pre-treatments of seeds with chemicals followed by acceletated ageing treatment significantly arrested the leakage of soluble substances and checked the declining of the levels of some vital cellular components like carbohydrate, protein, RNA and dehydrogenase enzyme and of the percent TTC-stained seeds.

It has been documented in different literatures that there were numerous varieties of aromatic rice available in Himalayan region. But, due to wrong methods of collection, unscientific storage system and introduction of high Yield variety, the indigenous varieties started to loss their existence in their own natural habitat. Field visit recorded that more than twenty-one different aromatic rice varieties are available in the Darjeeling district in the high land areas. In this investigation an attempt was made to check the potential of rice seeds which undergo adverse storage environment. Thus the major, objective of this work was to test the efficacy of growth regulators on the alleviation of seed under adverse storage.

2. Materials and Methods

Plant materials: Local aromatic rice (*Oryza sativa* L.) of 4 varieties (Krishnabhog, Musli, Kattaka and Dhanasae) collected from Darjeeling, West Bengal, India. After collection, the seed lots were separated from husk and healthy, undamaged seeds were used for experimental purposes.

During the experimental period the environmental conditions of Darjeeling were as follows: Temperature: 20-22° Celsius, Relative humidity: 95±5%.

Seed Pre-treatment: After the surface sterilization with 0.1% mercuric chloride ($HgCl_2$) for 90sec., all the seed varieties were separately pre-soaked with aqueous solutions of 100,200 and 500µg/ml of GA_3 or distilled water for 8 hours and then dried back to original weight of seeds. After 8 hours intervals such soaking and drying treatments were repeated 3 times to make the total duration of pre-treatment of 24 hours. This mode of pre-treatment enabled maximum penetration of the chemical while avoiding the commencement of germination. After complete pre-treatment of seed lots, the pre-treated seed lots (20g) each were put into separate muslin cloth bags and thus stored in a desiccators in which an environment of 98.2% relative humidity was pre imposed by keeping 250 ml 5.96% sulphuric acid (vol/vol) within it. This experimental setup was kept allowing the seeds to experience forced ageing treatment and sulphuric acid was changed periodically to restore the desired relative humidity within the dissector throughout the experimental period. Starting from 0- day, analyses were made at 10-days intervals upto 30 days after imposition of accelerated ageing condition, and then the experiment was terminated.

Biochemical analysis: The soluble and insoluble carbohydrate levels from the seed kernel were analysed, following the method of McCready et.al (1950). The activity of total dehydrogenase of intact seeds was analysed by the reaction of TTC according to the method of Rudrapal and Basu (1979). Protein and free amino acids levels of seed kernels were analysed by following the methods described by Lowry et.al. (1951) and Moore and Stein (1948). The seed enzyme activity, particularly, alpha amylase was extracted and estimated from the pre-treated seed lots following the method described by Biswas and Choudhuri (1978). All the data were statistically analysed at the treatment and replication levels (Panse and Sukhatme, 1967).

3. Results and discussion

Deterioration of seeds is a natural catabolic process which terminates their life span resulting in loss of viability. This process may be accelerated by some pathogenic attack or by adverse environmental conditions. This inevitable detrimental processes leading to deterioration of seeds is a matter of concern to the seed physiologists and crop growers all over the world. The problem of retention of seed vigour in Darjeeling and surrounding areas of India is more acute because of extremely high RH which is conducive to the growth of micro-organisms resulting in expeditious deteriorations of seeds.

Efficacy of several classes of chemicals viz., hormones, retardants, redox chemicals, phenols, vitamins and some salts on maintenance of seed health under storage has been reported. GA_3 is one of the most well established plant growth hormone which played an important role in seed biochemical and physiological changes. The chemical is quite promising in manipulation of physio-biochemical activities horticultural plants.

The germinability and the metabolic activities of the rice seeds declined with increased duration of accelerated ageing. In this experiment, decline in metabolism occurred at a higher rate for seeds treated with plant growth regulators. The effect of seed treatment with distilled water or GA_3 with three different concentrations (50,150 and 300 µg/ml) was found to be significant in metabolism like soluble and insoluble carbohydrate (Fig.1 and 2), Protein and free amino acids (Fig. 3 and 4), dehydrogenase and amylase activity increased with time of ageing, were found to be increased in case of chemical treated samples (Fig.5 and 6).

In the short term accelerated ageing period, the high RH (relative humidity) rapidly enhanced the rate of ageing and senescence of seeds and viability. The results are indicative of the fact that accelerated ageing caused to damage seed membrane which consequently resulted in the higher leakage of soluble carbohydrate in both chemical pretreated and non chemical pretreated seeds. Leaching of soluble and insoluble carbohydrate (table-1& 2) and free amino acids (table-4) from

the accelerated ageing storage seeds showed positive result with the increase period of accelerated ageing. Data showed that the 0-day after ageing GA_3 at its highest concentration significantly increased leaching in both treated and non-treated samples. Accumulation of amino acids in seed leachates went on increasing in all seed samples with advancement of agei ng duration. The same trend was apparent in case of chemical-pre treated seed samples. Unlike the changes of leakage products, protein contents (table-3) of seed kernels gradually declined keeping pace with days of accelerated ageing in non-chemical pre-treated seed samples. But in pre treating chemical sample leaching protein level goes on increasing. Total dehydrogenase activities of seed samples (table- 5) observed that the gradual increase and then slight fall with advancement of ageing duration. In case of alpha amylase (table- 6) effect remained almost constant in both GA_3 pre treated and non chemical pre treated samples.

The above results, therefore, point out that deterioration is a common phenomenon both in GA_3 treated and in non-chemical treated seed samples. The catabolic processes within the treated seeds remained somewhat same as control, thereby inefficient in tolerant against unfavourable storage environments. The results of this investigation showed that high RH treatment enhanced the ageing process of rice seeds as evident by analysing different reliable biochemical parameters used in this investigation. The chemical reduced carbohydrate, protein, free amino acids rapidly from aged seeds. In this investigation, the observed chemical induced rapid loss of metabolic activities under accelerated ageing condition, are indicative of the fact that GA_3 helped in growth and development but ineffectual to tolerate the unfavourable storage environment. There are numerous reports seed membranes are highly affected in deteriorating seeds, resulting in increased permeability and decreased germination of seeds. Thus, it seems that the chemical used in this investigation have no potential for retaining membrane integrity for certain duration.

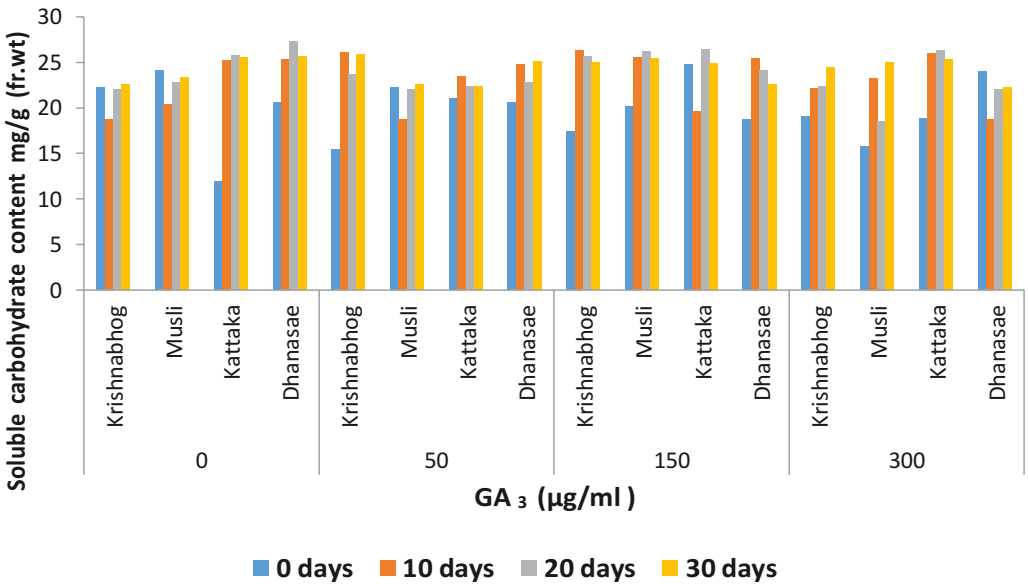

Figure 1: Effect of GA_3 (50, 150 and 300µg/ml) or distilled water (0) on changes in Soluble carbohydrate content (mg/g fr.wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae.

Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively.

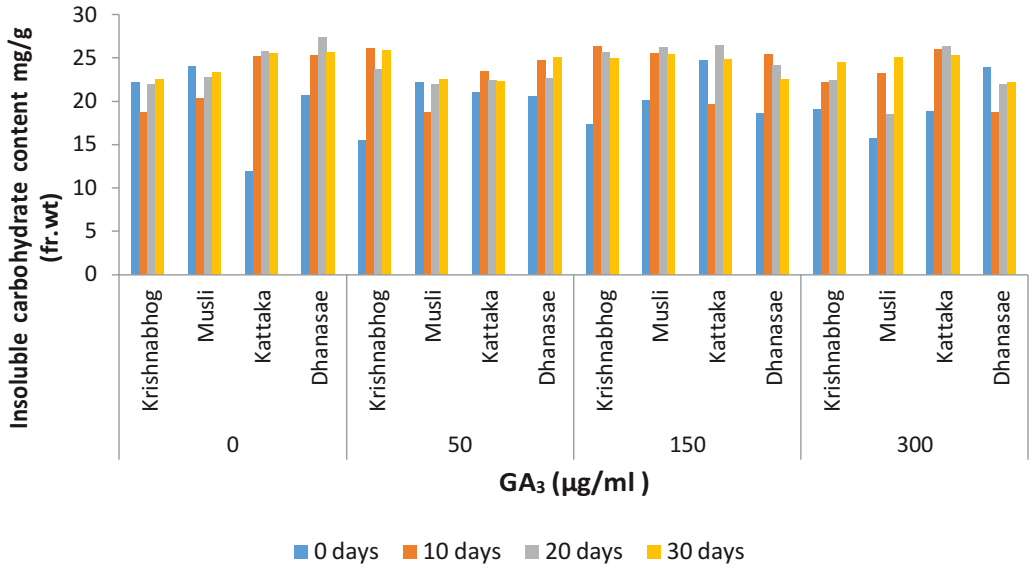

Figure 2: Effect of GA_3 (50,150 and 300µg/ml) or distilled water (0) on changes in Insoluble carbohydrate content (mg/g fr.wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae

Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively.

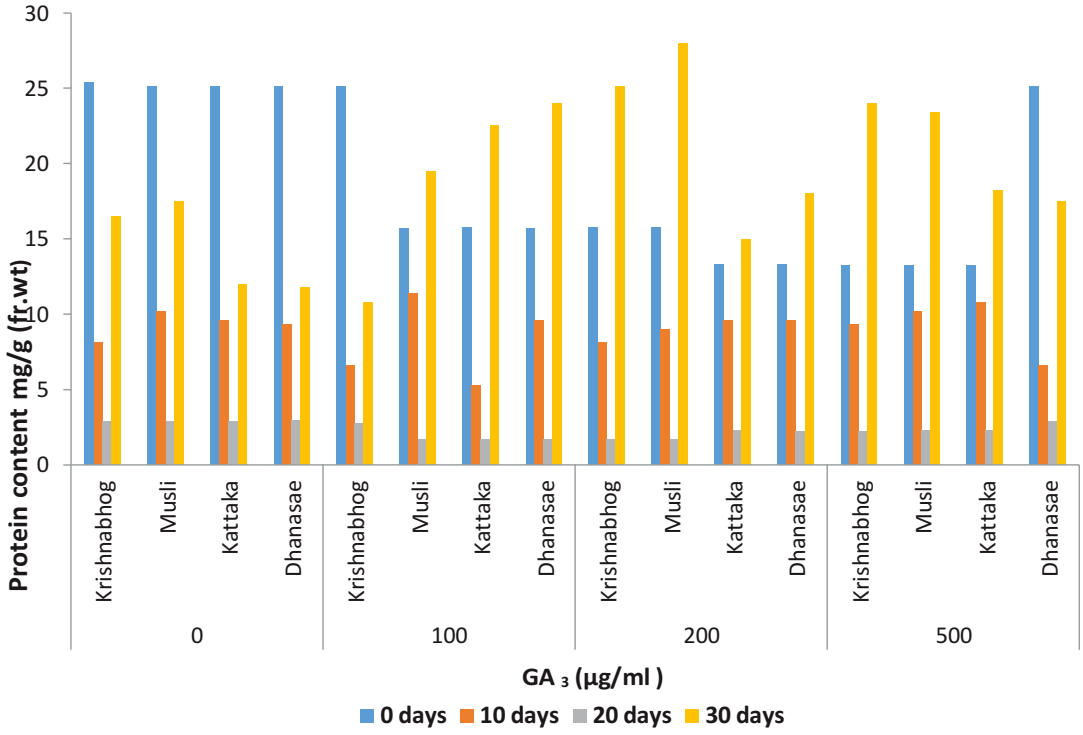

Figure 3: Effect of GA_3 (50,150 and 300 µg/ml) or distilled water (0) on changes in Protein content (mg/g fr.wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae

Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively.

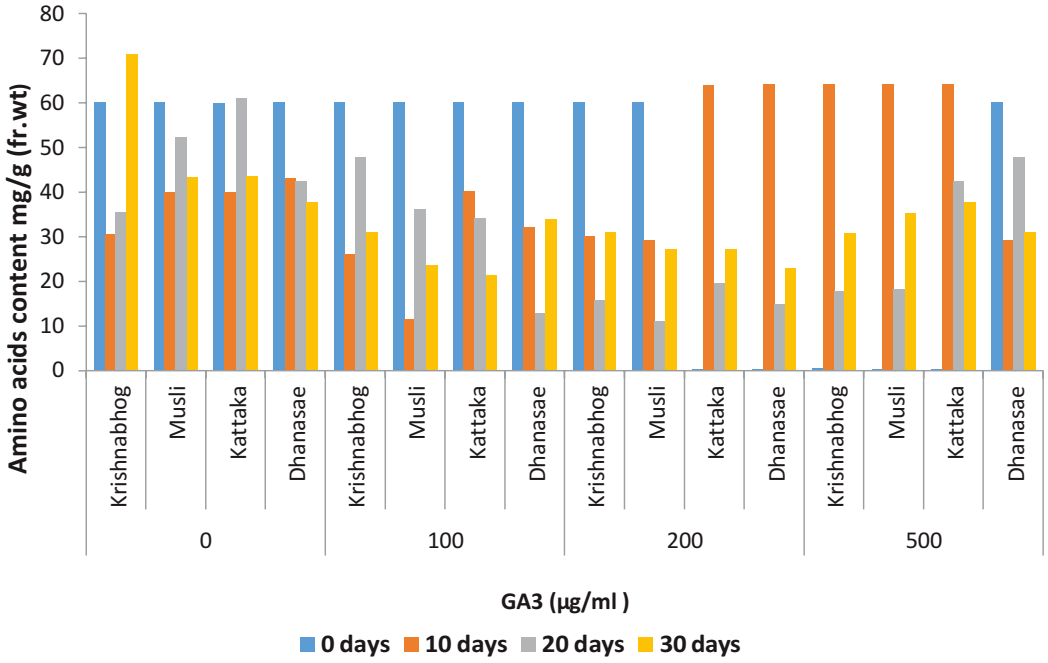

Figure 4: Effect of GA_3 (50,150 and 300 µg/ml) or distilled water (0) on changes in Amino acids content (mg/g fr.wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae.

Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively.

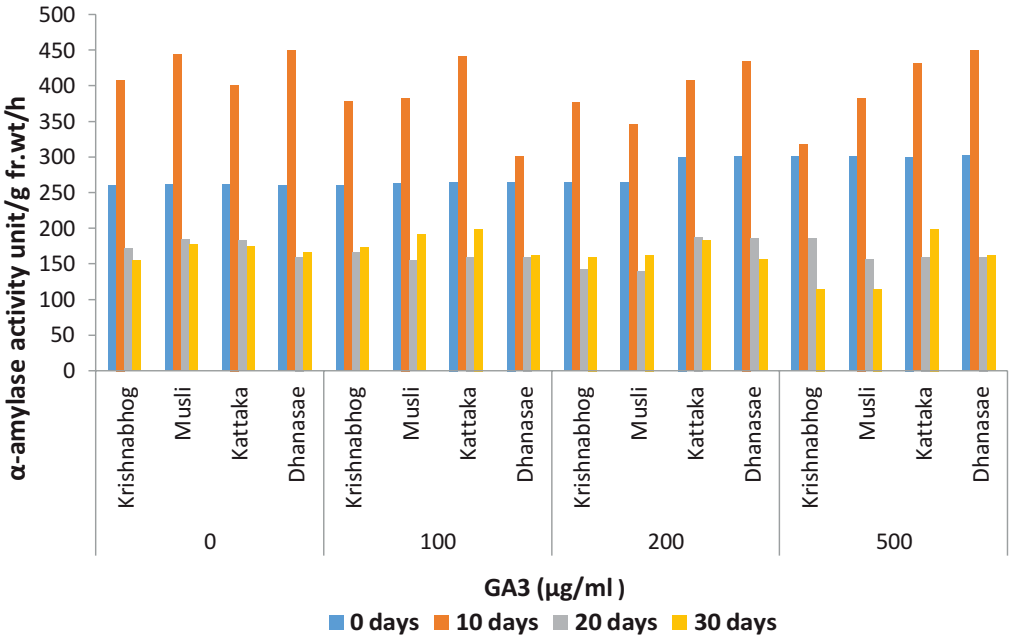

Figure 5: Effect of GA_3 (50,150 and 300 µg/ml) or distilled water (0) on changes in α-amylase activity (unit/g fr.wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae.

Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively.

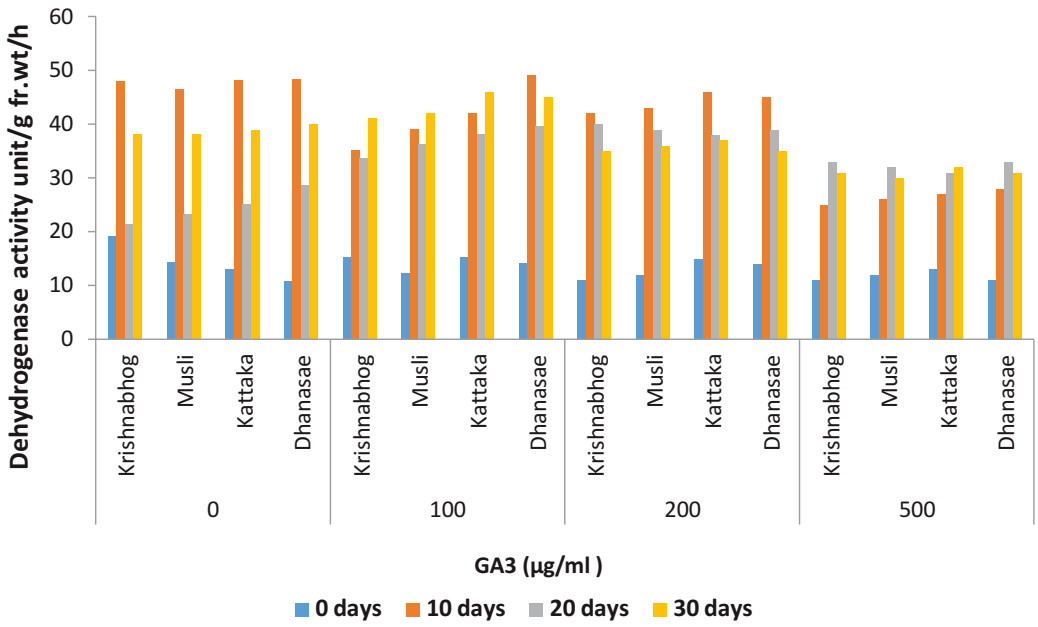

Figure 6: Effect of GA_3 (50,150 and 300 µg/ml) or distilled water (0) on changes in Dehydrogenase activity (unit/g fr.wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae.

Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively.

4. Acknowledgement

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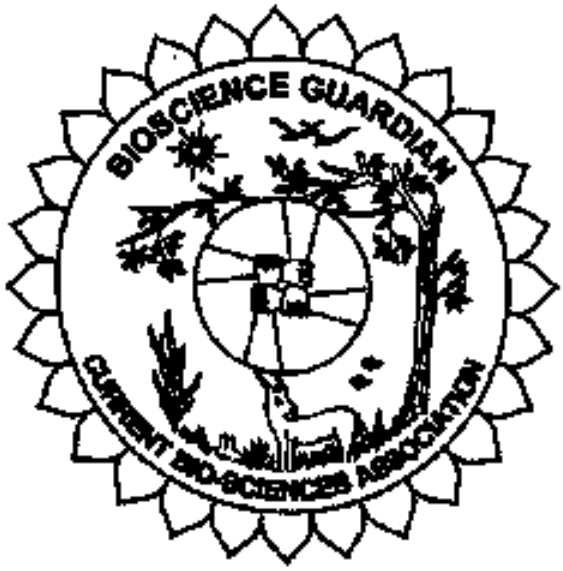

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EFFECT OF SODIUM-DIKEGULACON MAINTENANCE OF VIABILITY OF AROMATIC RICE SEEDS UNDER ADVERSE STORAGE CONDITION

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ABSTRACT

Aromatic rice occupies a prime position in Indian culture, not only because of their high quality, but they have been considered auspicious. Most of the traditional aromatic rice varieties are low yielding. Accelerated ageing treatment (98.2% relative humidity, RH) on four highland varieties of aromatic rice seeds (Krishnabhog, Musli, Kattaka and Dhanasae) for 30-days reduced the vigour of germinability and increased the leaching of sugar and amino acids with progress of ageing duration. Such deleterious changes were associated with proportional reduction in the level of protein, insoluble carbohydrate and the total activity of total dehydrogenase & α-amylase activities. Pre-treatment of rice seeds with Sodium-Dikegulac (Na-DK 2, 3:4-6-di-O-iso-propylidene-α-L-xylo-2-hexalofuranosate) significantly arrested the declining of levels of cellular components such as carbohydrate, protein and enzyme activities and also germinability of the seeds.

Keywords: Aromatic rice, growth retardant, Sodium-Dikegulac.

INTRODUCTION

India contributes about ⅓ of the world acreage under rice cultivation. Rice (*Oryza sativa* L.) the staple food of nearly one-half of world's population, contributes over 20% of the total calorie intake of man. India is one of the worlds, second only to China, cultivating 43 million hectares annually, which is about a third of the World acreage under rice. Rice (*Oryza sativa* L.) is the major cereal crop of India, being available in over 50,000 varieties differing with respect t. size, texture glutinous nature, and aroma and cooking quality.

India had an immense wealth of aromatic rice. In the Indian subcontinent, aromatic rice is categorized as Basmati & non-Basmati (Singh *et. al*, 2000). The Basmati types are characterized by long, slender grains having kernel length of 6 mm and more, length (L) to breadth (B) ratio (L/B ratio) of 3 and above, & high kernel elongation after cooking.

The grains of Basmati cultivars are pointed at both ends with gradual tapering at the end opposite to the germination end and have uniform breadth between the tapering (Mahindru, 1995). The non-Basmati aromatic rice also has one or more of the Basmati characteristics, but not all them.

Especially, they have small and medium kernel length, although they may have similar L/B ratio or kernel elongation rate as high as Basmati or more. But overall kernel elongation after cooking is much higher in Basmati types than in non-Basmati types. Aromatic rice is traditionally grown in the Himalayan foothill regions of India and Pakistan.

Seed deterioration is a natural catabolic process which may be accelerated by some pathogenic attack and /or by adverse environmental conditions. The problem of retention of seed vigour & viability in Darjeeling & surrounding areas of India is more acute because of extremely high RH which is conducive to the growth of microorganisms resulting in expeditious deterioration of seeds. As most crop seeds undergo post-harvest storage for either one or several planting seasons, agriculturists and horticulturists of this region are often handicapped by non-availability of standard vigour seed for healthy seedling production. Considering this problem of seed storing in the hilly region of Darjeeling, an attempt was made in this investigation to prolong the storage life of aromatic rice seeds having viability problems with chemical manipulations. There are several classes of chemicals like hormones, retardants, redox chemicals, phenols, vitamins etc. which plays significant role in seed vigour & viability maintenance. Na-Dikegulac, a retardant, shows effective role on seed viability & plant senescence, the present investigators tested the possibility of this chemical on the regulation of seed senescence of aromatic rice under adverse storage conditions.

MATERIALS AND METHODS

Plant materials: Darjeeling Hills Aromatic rice (*Oryza sativa* L.) of 4varieties (Krishnabhog, Musli, Kattaka and Dhanasae) were collected from the Principle Agricultural Office, Gorkhaland Territorial Administration, Darjeeling, West Bengal, India. All three varieties were identified by the Principal Agriculture Office itself. After collection, the seed lots were separated from husk and healthy, undamaged seeds were used for experimental purposes.

During the experimental period the environmental conditions of Darjeeling were as follows: Temperature: 20-22° Celsius, Relative humidity: 95±5%.

Seed Pre-treatment

After the surface sterilization with 0.1% mercuric chloride ($HgCl_2$) for 90sec., all the seed varieties were separately pre-soaked with aqueous solutions of 100,200 & 500µg/ml of Sodium-Dikegulac or distilled water for 8 hours and then dried back to original weight of seeds. After 8 hours intervals such soaking dry treatments were repea ted 3 times to make the total duration of pre-treatment of 24 hours.

This mode of pre-treatment enabled maximum penetration of the chemical while avoiding the commencement of germination. After complete pre-treatment of seed lots, the pre-treated seed lots (20g) each were put into separate muslin cloth bags and thus stored in a desiccators in which an environment of 98.2% relative humidity was pre-imposed by keeping 250 ml 5.96% sulphuric acid (vol/vol) within it.

This experimental setup was kept allowing the seeds to experience forced ageing treatment & sulphuric acid was changed periodically to restore the desired relative humidity within the dissect/throughout the experimental period. Starting from 0- day, analyses were made at 10-days intervals upto 30 days after imposition of accelerated ageing condition, and then the experiment was terminated.

Biochemical analysis

The soluble and insoluble carbohydrate levels from the seed kernel were analysed, following the method of Mc Cready *et. al* (1950). The activity of total dehydrogenase of intact seeds was analysed by the reaction of TTC according to the method of Rudrapal and Basu (1979). Protein and free amino acids levels of seed kernels were analysed by following the methods described by Lowry *et. al.* (1951); Moore and Stein (1948). The seed enzyme activity, particularly, alpha amylase was extracted and estimated from the pre-treated seed lots following the method described by Biswas and Choudhuri (1978). All the data were statistically analysed at the treatment and replication levels (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

The pathogenic attack and high relative humidity in the environment are inevitable detrimental processes leading to deterioration of seeds and it is a matter of great concern to the seed physicologists and crop growers all over the world. The problem of retention of seed vigour in Darjeeling and surrounding areas of India is more acute because of extremely high RH which is conducive to the growth of micro-organisms resulting in expeditious deteriorations of seeds.

Efficacy of several classes of chemicals viz., hormones, retardants, redox chemicals, phenols, vitamins and some salts on maintenance of seed health under storage has been reported. Bocion *et. al* first observed that sodium Dikegulac an intermediate product in the commercial synthesis of L-ascorbic acid, acts as a plant growth regulator. The literatures suggested that this chemical is quite promising in manipulation of physio-biochemical activities in horticultural plants.

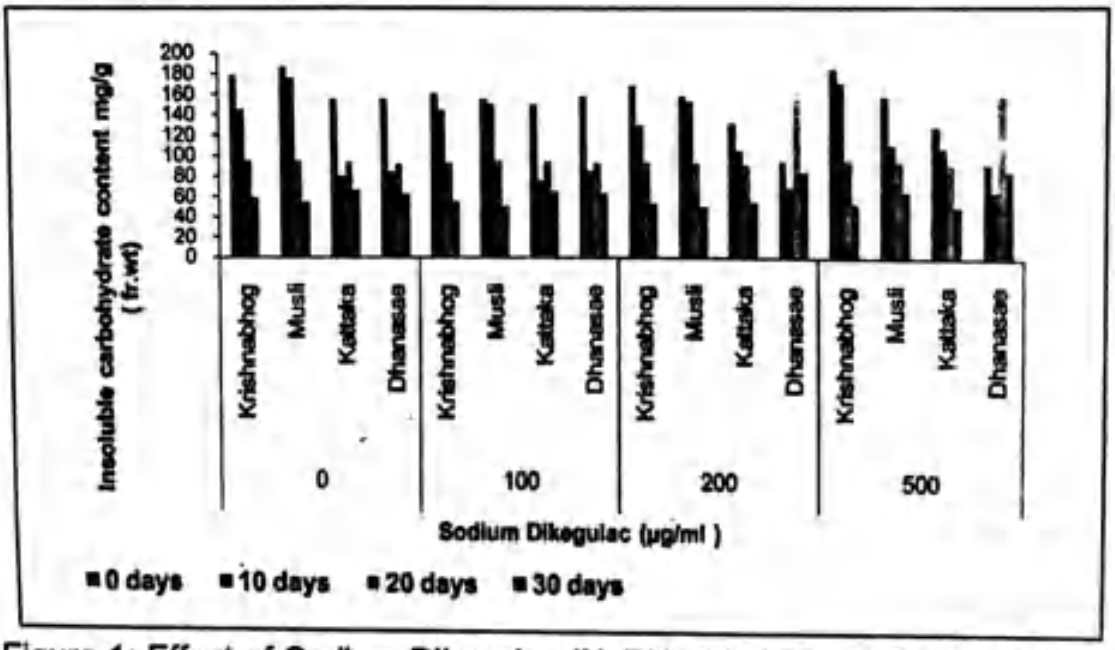

Figure 1: Effect of Sodium-Dikegulac (NaDK 100, 250 and 500 µg/ml), on changes in Insoluble carbohydrate content (mg/g fr.wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae. Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively.

The germinability & the metabolic activities of the rice seeds declined with increased duration of accelerated ageing. However, this decline in metabolism occurred at a slow rate for seeds treated with plant growth regulators.

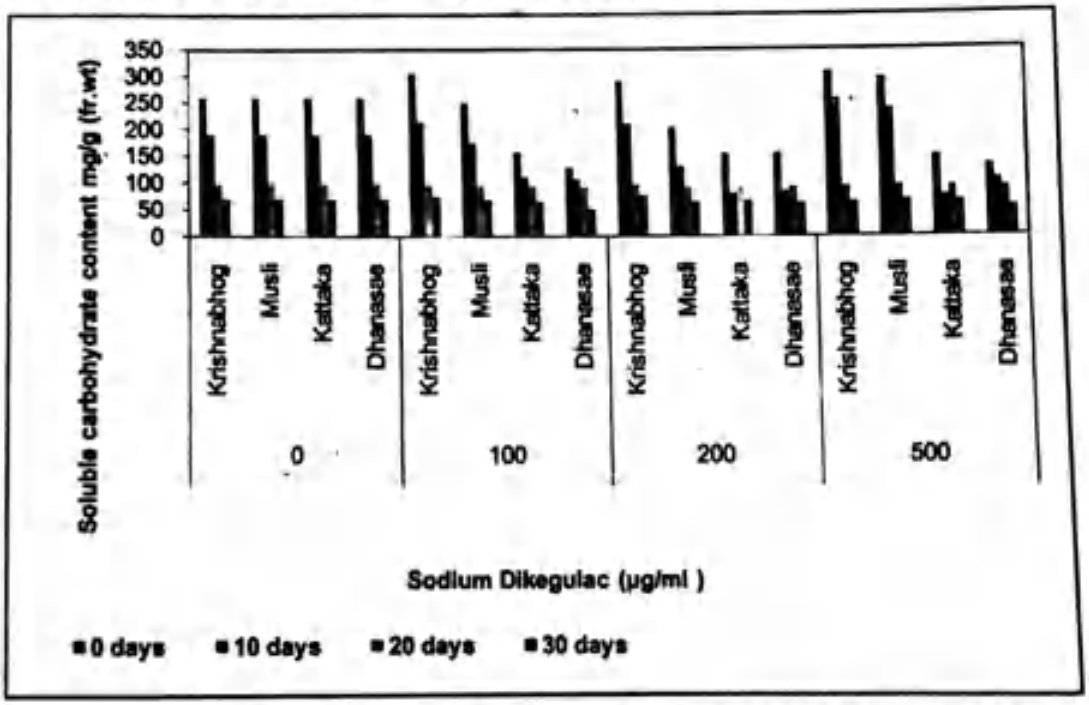

Figure 2: Effect of Sodium-Dikegulac (NaDK 100, 250 and 500 µg/ml), on changes in Soluble carbohydrate content (mg/g fr.wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae. Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively.

The effect of seed treatment with distilled water or Na-DK with three different concentrations (100, 200 & 500 $\mu g/ml$) was found to be significant in metabolism like soluble & insoluble carbohydrate (Fig 1 and 2), Protein and free amino acids (Fig 3 and 4). The dehydrogenase & amylase activity increased with time of ageing, were effectively arrested by growth regulators (Fig 5 and 6).

The results of this investigation showed that high RH-treatment enhanced the ageing process of rice seeds as evident by analysing different reliable biochemical parameters used in this investigation. Pre-treatment of the seeds with different growth regulators during the storage period significantly reduced the loss of germinability over untreated control samples.

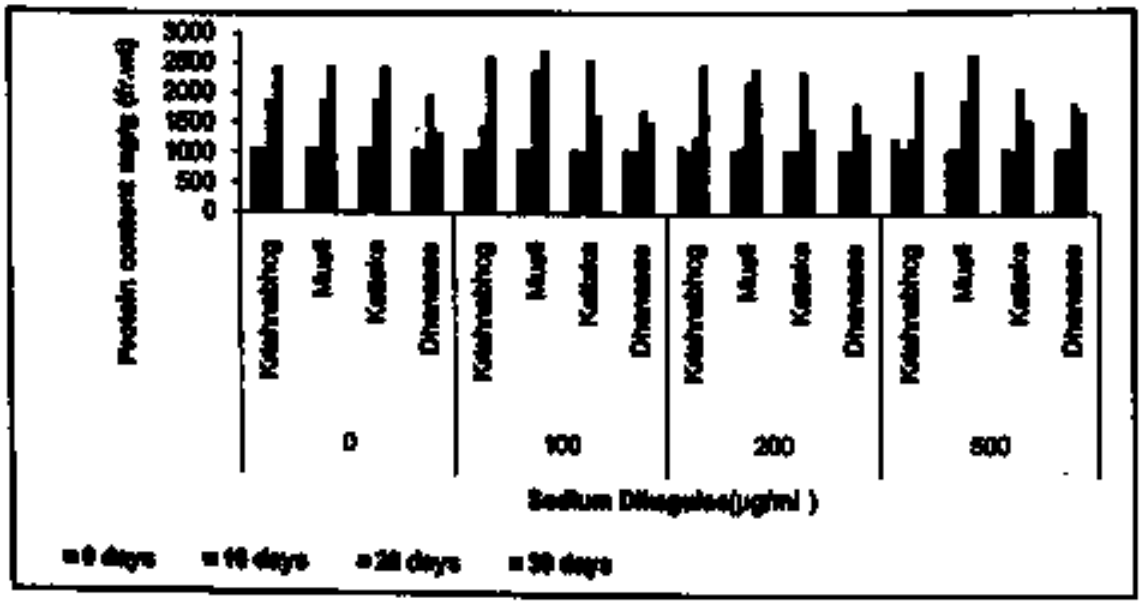

Figure 3: Effect of Sodium-Dikegulac (NaDK 100, 250 and 500 µg/ml), on changes in Protein content (mg/g fr. wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae. Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively

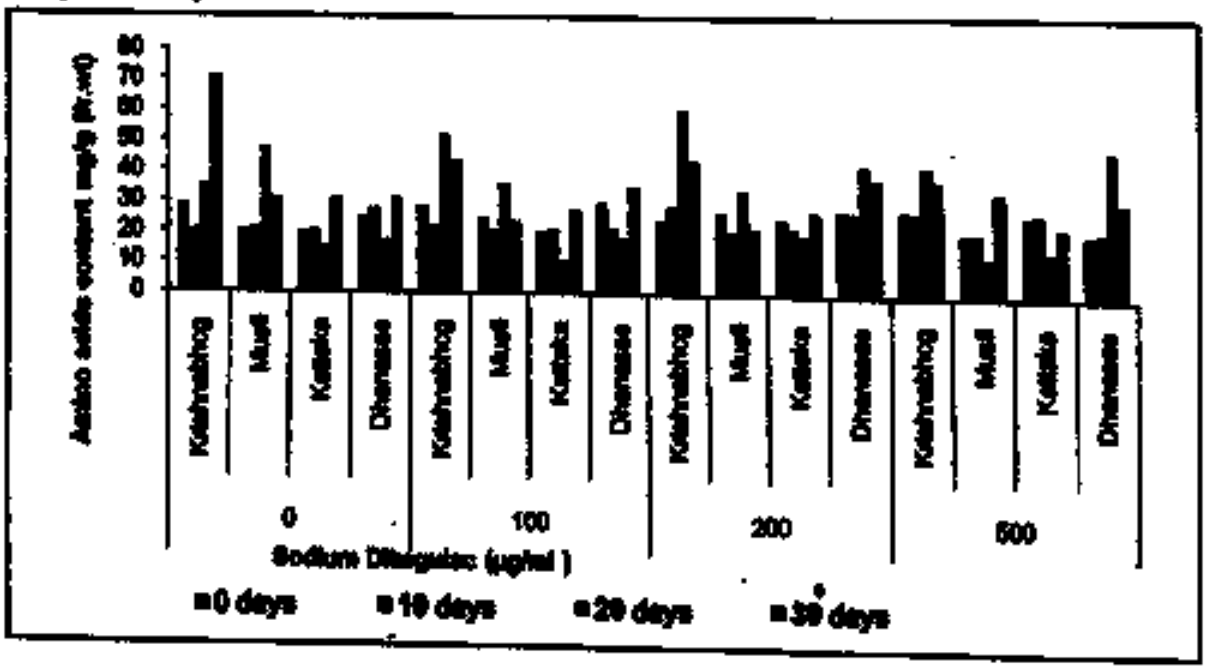

Figure 4: Effect of Sodium-Dikegulac (NaDK 100, 250 and 500 µg/ml), on changes in Amino acids content (mg/g fr. wt) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasae. Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10- 20- and 30-days respectively.

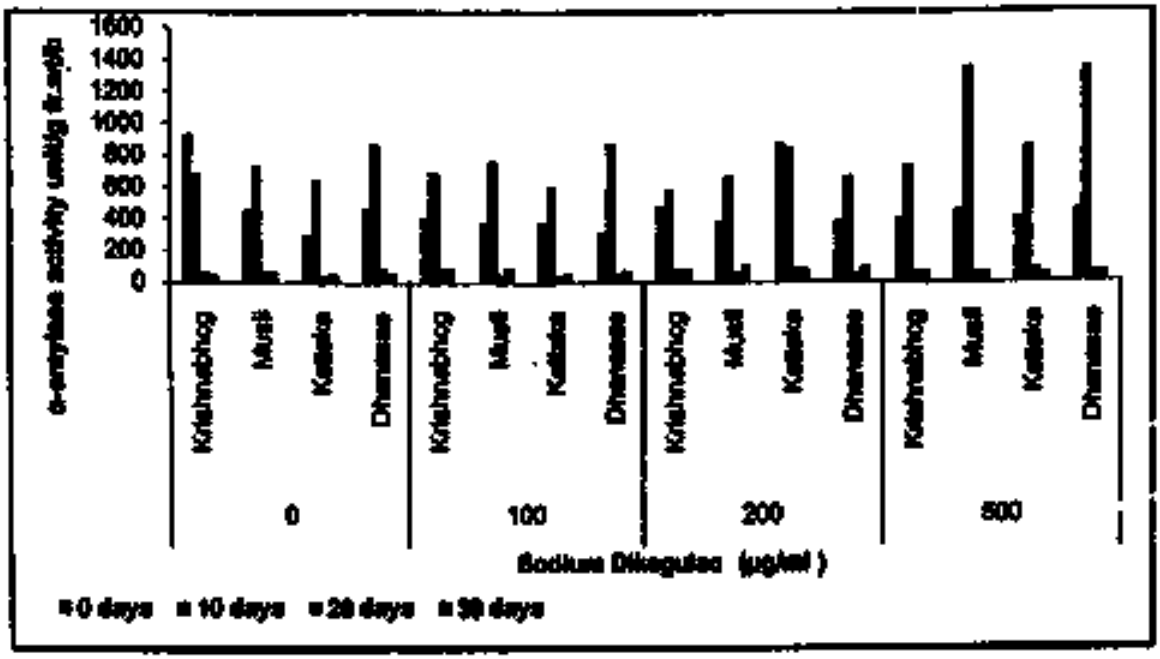

Figure 5: Profile of α-amylase activity with pre-treatment of Sodium-Dikegulac (NaDK 100, 250 and 500 µg/ml) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasee. Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded at an interval of 0-, 10-, 20- and 30-days respectively.

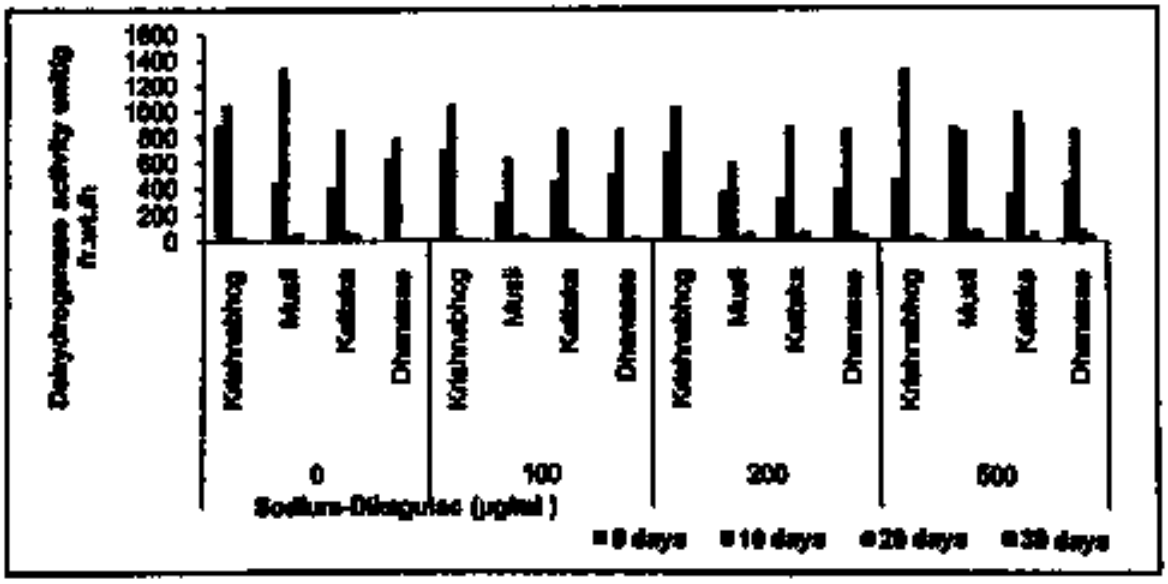

Figure 6: Profile of Dehydrogenase with pre-treatment of Sodium-Dikegulac (NaDK 100, 250 and 500 µg/ml) in seeds of aromatic rice varieties Krishnabhog, Musli, Kattaka and Dhanasee. Mature and healthy seeds were pre-treated with the chemicals and distilled water for 24 hours. Data were recorded of an interval of 0-, 10-, 20- and 30-days respectively.

The chemical reduced carbohydrate, protein, free amino acids from the rapidly aged seeds. In this investigation, the observed

chemical induced alleviation of the rapid loss of metabolic activities under accelerated ageing condition, are indicative of the fact that Na-DK helped the seeds to tolerate the unfavourable storage environmen t. There are numerous reports seed membranes are highly affected in deteriorating seeds, resulting in increased permeability & decreased germination of seeds. Thus, it seems possible that the chemicals used in this investigation have some potential for retaining membrane integrity at least for certain duration.

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