NBUJPS
NBU Journal of Plant Sciences

**ISSN No.** 0974-6927
Vol. 14 (2022), pp. 8-20

*Research Article*

# Study of Major Isoflavones in Mungbean Seedlings with Special Emphasis on Its Enhanced Antioxidant Activity After Solid Matrix Priming with Selected Elicitors Including Nano-Chitosan Under Salinity Stress

***Sujoy Kumar Sen*[1, 2, 3], *Palash Mandal*[2], *Jnan Bikash Bhandari*[3*]**

[1]Department of Botany, Siliguri College, Darjeeling, West Bengal, India-734 001
[2]Nanobiology and Phytotherapy Laboratory, Department of Botany, University of North Bengal, Darjeeling, West Bengal, India-734 013
[3*]Pteridology and Palaeo Botany Laboratory, Department of Botany, University of North Bengal, Darjeeling, West Bengal, India-734 013

## Abstract

For a long time, mung bean has been a well-liked crop. It is frequently used as a popular dish in the primarily cereal-based diets of Asian countries for its physiological functionalities, such as antioxidant, antitumor, and antidiabetic activities. Isoflavones present in legume-based foods have high antioxidant potential. These isoflavones are considered beneficial to human health and are linked to a reduced risk of cardiovascular disease, osteoporosis, and the prevention of certain types of cancer in humans, including breast, prostate, and colon cancer, as well as menopausal symptoms. On the other hand, nanotechnology is starting to look like an excellent method to boost food production and make farming less hazardous to the environment. Fascinatingly, the seed nano-priming method demonstrated promising results to mitigate the detrimental effects of different abiotic stress factors including salinity stress on crop plants and has thus, led to higher crop yields. The current study aimed to evaluate the effects of solid matrix priming (SMP) using nano-chitosan in mung bean sprouts under salinity stress related to the production of major mung bean isoflavones, which were detected through high-resolution liquid chromatography-mass spectrometry. When compared to unprimed seedlings exposed to salinity stress conditions, phytochemical quantification showed that SMP with nano-chitosan showed improved antioxidant activities as well as the highest total flavonoids and proline content. Under salinity stress, SMP with nano-chitosan significantly increased the biochemical anti-oxidative properties in germinated mung bean seeds, and also provided salt tolerance. As a familiar healthier choice, and because of the significance of mung bean sprouts for human health and the industry's rapid expansion, nutritional enrichment of this food has emerged as a significant field of study.
.

***Article info***

Received **19 December 2022**
Revised **22 April 2023**
Accepted **28 April 2023**

**Keywords:** Mung bean sprouts; Isoflavones; Nano-chitosan; Salinity; Solid matrix priming

**DOI:** *https://doi.org/10.55734/nbujps.2022.v14i01.002*

## Introduction

Legumes from the Fabaceae family are the primary source of isoflavones (Dixon and Sumner 2003) and mung bean [*Vigna radiata* (L.) R. Wilczek] has long been a popular leguminous crop that is frequently used as a popular dish in the predominantly cereal-based diets of Asian countries and accepted all over the world for its physiological functionalities, such as antitumor, antioxidant, and antidiabetic activities (Li et al. 2012; Yao et al. 2013). It is a well-balanced source of vitamins, minerals, fiber, protein, and bioactive compounds in significant amounts (Gan et al. 2017). According to studies, mung beans have physiological properties that include anti-obesity,

* ***Correspondence*** - jnanbikashbhandari@nbu.ac.in

anti-oxidation, and antibacterial effects (Yao et al. 2013). In many developing nations, mung beans offer a high-quality, all-natural plant protein source that has been utilized in place of meat and dairy products (Connolly et al. 2015; Du et al. 2018). Mung bean polysaccharide has been shown in studies to have antioxidant and immunoregulatory properties (Lai et al. 2010). Additionally, the mung bean's essential fatty acids can aid in the body's growth and development. Mung beans are a significant source of phytochemicals, such as phenols and flavonoids, which have health-promoting properties like antioxidants, anti-tumor, and radiation protection, in addition to their nutritional value (Randhir and Shetty 2007; Soucek et al. 2005). Nanotechnology in the form of seed nano-priming is an emerging field of study and intriguingly, the seed nano-priming strategy has been displayed to be effective against various abiotic stress factors, and it has also increased yields of crops (Abbasi Khalaki et al. 2021; Ye et al. 2020). Among the various seed priming techniques, solid matrix priming (SMP) is an efficient and less frequently used innovative method which is quite advantageous over liquid or osmotic priming (Sen & Mandal 2018). In SMP, wet seeds are combined with a solid substance and their moisture content is adjusted lower than that needed for seed sprouting (Harman & Taylor 1988). Seed priming, which involves applying nanoparticles to seeds to stimulate germination and subsequent plant growth by triggering the physiological functions of plants and giving resistance to various stresses, is a novel and cost-effective method. Priming seeds with nanoparticles stimulates electron transfer and boosts the capacity of the seeds' surfaces to react to substances found in the cells and tissues of the plant. By Seed nano-priming, the equilibrium between plant growth hormones and reactive oxygen molecules can be maintained and the biochemical processes can also be controlled (de Espirito Santo Pereira et al., 2021). Chandrasekaran et al. (2020) found that when nanoparticles are taken up by the seed coating, they might boost the generation of reactive oxygen species, work through various metabolic processes, raise the amount of active gibberellins, and get proteins out of storage. Additionally, the increased water intake by the seeds as a result of nanoparticles' effects can stress the seeds enough to trigger germination and boost the activity of several enzymes throughout phases I and II of the germination process (Joshi et al. 2018). Due to the enhanced activity of enzymes including superoxide dismutase, catalases, and guaiacol-peroxidase under stress circumstances, nanoparticles can operate to lower seed ROS levels, minimizing seed damage (Guha et al. 2018). Since seed sprouting is the first step in improving a crop, it could be used as a way to measure whether nanomaterials are beneficial or detrimental to the crop (Ahmed et al. 2019). Several genes, including those involved in the plant's tolerance to stress, have recently been shown to be triggered by seed nano-priming when sprouting is in progress (An et al. 2020; Ye et al. 2020). Plant-based foods containing isoflavones are thought to have high antioxidant potential and be good for human health. Isoflavones are one of four plant-based phenolic substances, along with stilbene, coumestan, and lignan, that fall under the category of phytoestrogens, which are estrogen-like compounds, structurally similar to 17-estradiol and can bind to estrogen's alpha and beta receptors (Cornwell et al. 2004; Sirotkin and Harrah 2004). The roles of the alpha and beta estrogen receptors vary. While beta estrogen receptors are in charge of cell apoptosis, alpha estrogen receptors are involved in cell proliferation (Rietjens et al. 2013). Due to their ability to block intracellular signaling pathways linked to NF-kappa B and immune responses, phytoestrogens have an impact on the immune system. Specific immune reactions and lymphocyte proliferation can be inhibited by genistein (Jefferson and Williams 2011). Additionally, isoflavones have the same antioxidant, anticancer, antimicrobial, and anti-inflammatory properties as other flavonoids (Conklin et al. 2007; Dhayakaran et al. 2015; Rodríguez-Roque et al. 2013; Chacko et al. 2007). Isoflavones may be able to scavenge free radicals, reduce low-density lipoprotein and DNA susceptibility to oxidative stress, and increase the activity and expression of antioxidant enzymes, according to the research on their antioxidant effects (Erba et al. 2012). In plants, phytoestrogens do not function as hormones, but as phytoalexins, synthesized and accumulated in plants during stress and microbe attacks. These active defense compounds have fungistatic, antibacterial, antiviral, and antioxidant properties (Dakora and Phillips 1996). They also prevent angiogenesis, thereby being important in the fight against malignant tumors (Bellou et al. 2012). In plants, isoflavonoids play many roles in plant-microbe interactions, including rhizobia-legume symbiosis and defense responses (Rípodas et al. 2013). In addition, they are also reported to be involved in abiotic stress responses. Their levels under stress depend on the

studied plant and stress type. Several studies have reported that UV-A and UV-B light have a positive influence on isoflavone accumulation (Liu et al. 2017), although some other stress reports are also not unambiguous. For example, in a study by Swigonska et al. (2014), after long and short-duration cold stress, osmotic stress, and combined cold and osmotic stress, the content of all the identified isoflavones such as daidzein, genistein, etc., increased in the roots of soybean seedlings. It is already reported that low-concentration salt stress could increase the total isoflavone content in chickpea sprouts (Gao et al. 2015). Similar results were also observed in tobacco and soybean plants with enhanced accumulation of the metabolites along with the beneficial impacts of isoflavones on plant salt tolerance (Jia et al. 2017).

In order to determine how solid matrix priming with nano-chitosan affects seedling vigor in mung beans subjected to salinity stress and how it affects the antioxidant potential of mung beans as well as the major mung bean isoflavones with health benefits for humans, the current study has been designed.

## Materials and methods

### *Collection and sterilization of mung bean seeds*

From the Pulse and Oilseed Research Station in Berhampur, West Bengal, India, a mung bean cultivar (SAMRAT) was procured. Previous research on salt stress sensitivity assessment by Sen et al. (2016) on five most popular mung bean cultivars of west Bengal viz, Samrat, Sonali, Panna, Sukumar, and Bireshwar, indicated that Samrat was the most salt tolerant cultivar. Hence, SAMRAT has been chosen for the current study. The seeds were surface sterilized for 3 to 5 minutes in 0.1% mercuric chloride ($HgCl_2$), followed by several rinses in sterile distilled water.

### *Preparation of nano-chitosan*

The well-known ionic gelation method was used to prepare nano-chitosan in the laboratory using low molecular chitosan and sodium tripolyphosphate (STPP) as a cross-linking agent (Rajeshwari et al. 2016). Chitosan powder was dissolved in 100 ml of a 2% acetic acid solution, weighing about 0.2g. After 15 minutes of stirring, the chitosan solution was thoroughly combined. The obtained chitosan solution was stirred continuously with a magnetic stirrer as the STPP solution, which had been prepared in distilled water, was added drop by drop. The setup was continuously stirred until the emulsion's milky color appeared. Notably, STPP is a safer ingredient when compared to other chemical cross-linkers.

### *Characterization of nano-chitosan*

Microstructural analysis of the morphology, shape, and size of the dried nano-chitosan sample was carried out using scanning electron microscopy (SEM) (Shende et al. 2014). Celite and the dried mixture of Celite-nano chitosan were both subjected to SEM. Zetasizer (ZETASIZER NANO ZS90 ZEN3690) was employed to investigate the size and distribution of the nano-chitosan particles using the Dynamic Light Scattering (DLS) method (Zaki et al. 2015). The structural characteristics of the particles were ascertained by Fourier Transform Infrared (FTIR) utilizing KBr pellets (Anusha and Fleming 2016).

### *Germination setup and sample preparation*

In separate airtight zipper bags (10.5 cm X 7.8 cm) containing 1g of celite (used as a matrix) that were kept at a 10% water content using nano-chitosan, sterilized seeds were appended for seed priming. Additionally, one more set of seeds was used as a control (i.e., unprimed). So in the current experiment, two treatments viz., chitosan primed and nano-chitosan primed seeds were used together with an unprimed control, with three replicates of each treatment. After 24 hours of priming, the seeds were taken out of the matrix (celite), dried, and stored refrigerated overnight. The seeds were maintained for seven days under 4 $dSm^{-1}$ salinity stress conditions while they germinated in the seed germinator (REMI) set to 25 ± 2°C. The saline solution's electrical conductivity was measured using a conductivity metre ($dSm^{-1}$ = Deci Siemen per metre). After that, a sample of the seedlings was prepared for biochemical tests.

### *Determination of proline content*

With a few minor modifications, the method outlined by Bates et al. (1973) was employed here to determine the free proline content in mung bean seedlings.

### *Determination of hydrogen peroxide ($H_2O_2$) content*

According to the procedure outlined by Loreto and Velikova (2001), hydrogen peroxide accumulation was measured.

***Determination of lipid peroxidation (malondialdehyde content)***

Malondialdehyde contents were calculated using the Davenport et al. (2003) recommended technique to estimate the extent of lipid peroxidation. The following formula was used to determine the amount of MDA present:

MDA content (µmol/g) = [6.45 ($A_{532}$ -$A_{600}$) – 0.56$A_{450}$] × $V_t$/W Where $A_{600}$, $A_{532}$, and $A_{450}$ represent absorbance at 600, 532, and 450 nm and $V_t$ = 0.0021 and W = 0.2 g.

***Determination of enzymatic antioxidant activity***

The mung bean samples (0.5 gm) were homogenized in a pre-cool mortar and pestle to assess the enzymatic antioxidant activity. The crushed sample was combined right away with 0.1 M ice-cold potassium phosphate buffer, which contains 0.5 mM EDTA and has a pH of 7.5 for catalase and superoxide dismutase and pH 6.8 for ascorbate peroxidase. The centrifuged supernatant after centrifugation at 15000 × g for 15 min at -10˚C from the crushed buffer sample from the centrifugation tube was used for the enzymatic assay.

***Detection of superoxide dismutase (SOD) activity***

The Esfandiari et al. (2007) method was used to estimate the activity of superoxide dismutase.

***Detection of catalase (CAT) activity***

The detection method prescribed by Aebi (1984) was followed for estimating *Catalase* activity with certain modifications.

***Detection of ascorbate peroxidase (APX) activity***

According to Chen and Asada's method (1989), ascorbate peroxidase activity was estimated. APX was expressed in terms of unit, where one unit was defined as the quantity of enzyme required to put away 1 µm of ascorbate $min^{-1}$ mg $protein^{-1}$.

***Determination of non-enzymatic antioxidant activity***

***Determination of ascorbic acid content***

Detection processes described by Omaye et al. (1979) were followed for estimating ascorbic acid content. Sample extraction was done by homogenizing 0.5 gm leaf samples in 10% (w/v) TCA, followed by centrifugation at 10,000 × g for 20 min at 25˚C to collect the supernatant. Detection was carried out by reacting 0.5 ml supernatant with 2% 2, 4-dinitrophenyl hydrazine in 0.5 N sulphuric acid and 10% thiourea. The absorbance of the reaction mixture was recorded at 520 nm after 3 hrs of incubation at 37˚C.

***Detection of flavonoid content***

The content of flavonoids was determined at 510 nm following the protocol of Atanassova et al. (2011) by adding 300 µl 5% $NaNO_2$, 300 µl 10% $AlCl_3$, and 2 ml 1 (M) NaOH to the aqueous extract and by using quercetin as standard. An assessment of the bioactive potential of primed seedlings of mung bean was conducted in terms of antioxidant activity.

***Quantitative profiling of free radical scavenging activities***

The antioxidant activity of mung bean seedlings was evaluated in terms of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay and $ABTS^+$ (2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) scavenging assay compared with appropriate standards. For extraction, the first 1 gm fresh mung bean sample was homogenized in 10 ml methanol with mortar and pestle and thereafter, centrifuged at 10,000 × g for 10 min at 4˚C. The method prescribed by Sidduraju et al. (2002), was followed for DPPH scavenging activity. DPPH scavenging activity was estimated at 517 nm by mixing 0.2 ml methanolic extract with 2 ml DPPH against ascorbic acid as standard. DPPH radicals scavenging activity was calculated as percentage inhibition according to the following equation: DPPH scavenging activity (%) = ($A_0$-$A_1$)/ $A_0$ × 100 Where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the sample. The final result of antioxidant activity (DPPH scavenging activity) was expressed as IC25 (µg/mL), which represented the sample concentration which represented the extract concentrations scavenging 25% of DPPH radicals (Nishaa et al. 2012). ABTS radical scavenging activity was estimated following the method of Sidduraju et al. (2002), against butyl-hydroxytoluene (BHT) as standard. ABTS antioxidant activity was estimated using butyl-hydroxytoluene (BHT) as standard. ABTS free radical scavenging activity was calculated as percentage inhibition according to the following equation: ABTS scavenging activity (%) = ($A_0$-$A_1$)/ $A_0$ × 100 Where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the sample. The final result of antioxidant activity (ABTS radical scavenging activity) was expressed as IC50 (µg/mL), which represented the extract

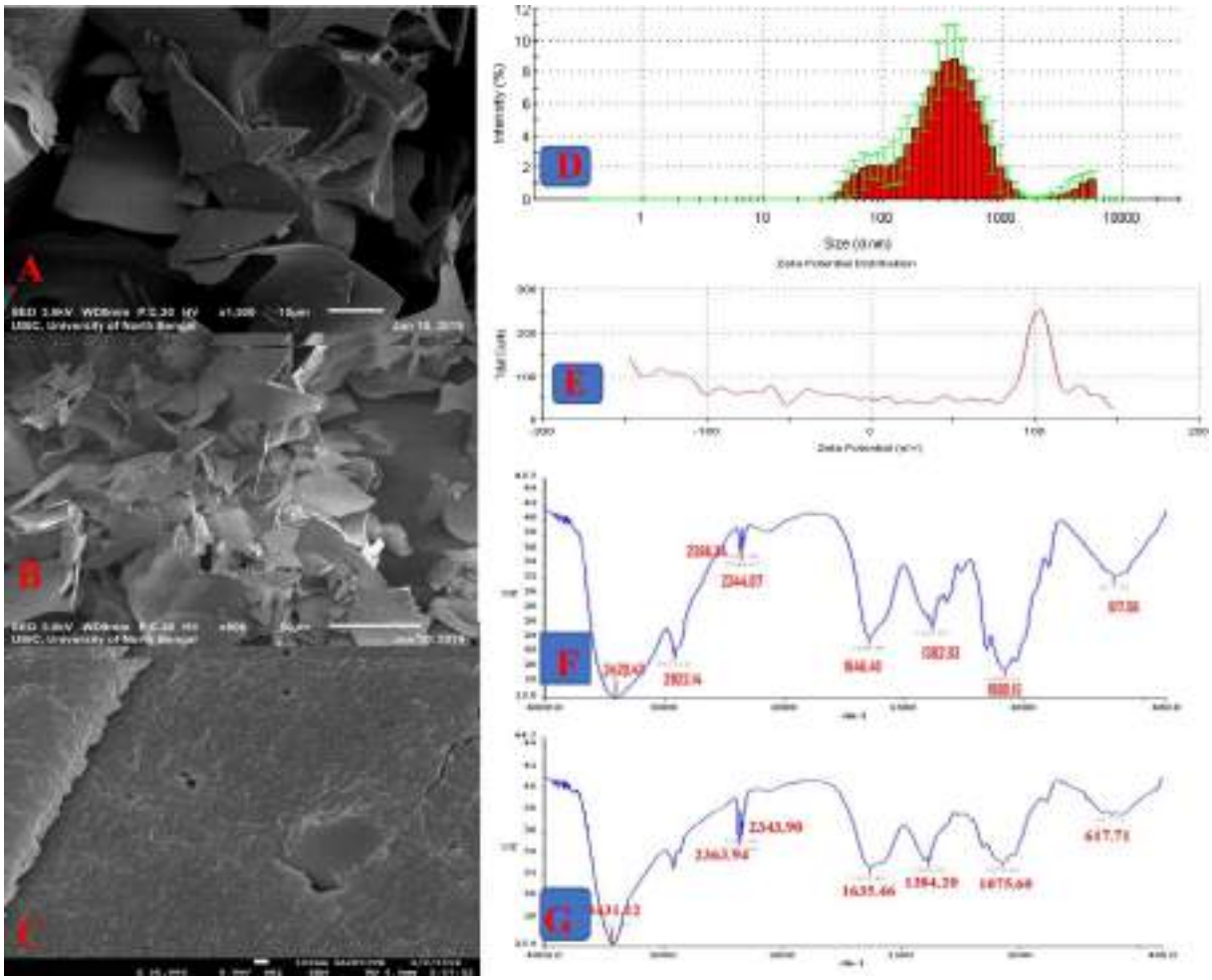


**Fig 1.** Characterization of nano-chitosan: (A) SEM of Celite, (B) SEM of Celite-nano-chitosan mixture, (C) SEM of nano-chitosan, (D) DLS of nano-chitosan, (E) Zeta potential of nano-chitosan, (F) FTIR spectra of chitosan and (G) FTIR spectra of nano-chitosan.

concentrations scavenging 50% of ABTS free radicals (Nishaa et al. 2012).

***Principal component analysis (PCA) and Heat map***

PCA and a heat map were created in this study using ClustVis Software and the heatmap R package (version 0.7.7). One of the methods in the pcaMethods R package is used to calculate the principal components.

***High-resolution liquid chromatography-mass spectrometry (HR-LCMS) analysis:***

HR-LCMS of untreated and treated mung bean seedlings were conducted from SAIF-IIT Bombay.

**Results and discussion**

***Scanning electron microscopy (SEM)***

**Fig.** 1A and **Fig.** 1B show scanning electron microscopy (SEM) of Celite and Celite-nano-chitosan mixture respectively. It is evident from the SEM image that celite particles are ordinarily very loosely arranged to have wide gaps between them while the SEM image of the Celite-nano-chitosan mixture shows a more compact arrangement. On the otherhand, **Fig.** 1C shows an SEM image of nano-chitosan, which highlights the amorphous nature of the nano-chitosan. It is a more or less uniform structure in contrast to chitosan powder which shows a non-porous, plain, and smooth structure in SEM as reported by Sudha et al (2014).

***Dynamic light scattering (DLS) and Zeta potential***

Numerous analytical tools with practical applications represent the distinctive modifications in nanoparticles that occur throughout their formation from the chitosan polymer. DLS optically quantifies the motion of the suspended particles as well as the size distribution of chitosan nanoparticles (Dubin 1967). The intensity of scattered light changes over time as a result of the constant, random Brownian motion of the chitosan nanoparticles in this particular dispersion. The poly-dispersity index (PDI) of the chitosan nanoparticles was determined to be 0.465 and the early correlation coefficient decay curve to be in the spectrum of 250 nm (**Fig.** 1D). In terms of size distribution, the chitosan-TPP binary electrolyte system under some circumstances consisted of prepared TPP cross-linked nano-chitosan. Chitosan nanoparticles were determined to have a zeta potential of +101 mV (**Fig.** 1E). The stability was greater with increased surface charge.

***Fourier transform infrared (FTIR) spectroscopy***

**Fig.** 1F and 1G represent the FTIR spectra of chitosan and nano-chitosan respectively. The intermolecular interaction of nano-chitosan is revealed by FTIR analysis. N-H, C-H, and O-H single bonds are responsible for the distinctive peak that is seen in the range of 4,000 to 2,500 in the spectrum of nano-chitosan. On the other hand, the recognisable peak in the nano-chitosan spectrum that appears between 2,500 and 2,000 represents triple bond absorption. In the spectrum of nano-chitosan, the distinctive peak can be seen around 2,000 and 1,500 and is caused by absorption from double bonds such C=O, C=N, and C=C. The strong and wide peak appearing around the 3420 $cm^{-1}$ area is attributed to hydrogen-bonded O-H stretching vibration in the chitosan spectra. The FTIR spectra of nano-chitosan exhibited a shift of the tip of the 3420 $cm^{-1}$ to 3431 $cm^{-1}$ which becomes wider with higher relative intensity in the FTIR of chitosan. This reflects the enhancement of hydrogen bonding. Moreover, at 1646 $cm^{-1}$ the peaks for N-H bending vibration of amine I and at 1382 $cm^{-1}$ the amide II carbonyl stretch shifted to 1635 $cm^{-1}$ and 1384 $cm^{-1}$ respectively. The presence of a P-O peak, which is clear from the FTIR measurements, also supported the relationship between the phosphoric and ammonium ions.

***Biochemical analysis***

***Isoflavone content and derivation of the mung bean Isoflavonoids (KEGG pathway):***

The group of secondary plant metabolites known as isoflavones is distinct and results from the phenylpropanoid pathway. They are mostly produced by Papilionaceae family members (Wang and Murphy 1994). **Fig. 2** displays the chromatograms obtained through quantitative LC-MS analysis of three major isoflavones namely (A) Biochanin-A, (B) Formononetin, and (C) Genistein which were found in mung bean sprouts.

The KEGG pathway of the flavonoid biosynthesis pathway could be used to explain how mung bean isoflavonoids are produced. As per the KEGG pathway, Genistein is derived from Naringenine via 2-Hydroxy-2, 3-dihydrogenistein ($C_{15}H_{12}O_6$). 2, 7, 4'-Trihydroxyisoflavanone acts as the substrate for the reaction that produces genistein, which is catalyzed by the enzyme 2-hydroxyisoflavanone dehydratase [EC: 4.2.1.105]. The enzyme isoflavone 4'-O-methyltransferase [EC: 2.1.1.212 2.1.1.46] then directly transforms genistein into Biochanin-A ($C_{16}H_{12}O_5$) (**Fig. 3**).

S-Adenosyl-L-methionine + Genistein <=> S-Adenosyl-L-homocysteine + Biochanin A

Genistein may also form Genistein 7-O-beta-D-glucoside ($C_{21}H_{20}O_{10}$), Prunetin ($C_{16}H_{12}O_5$), or 2'-Hydroxygenistein ($C_{15}H_{10}O_6$) by the enzyme isoflavone 7-O-glucosyltransferase [EC: 2.4.1.170], isoflavone-7-O-methyltransferase [EC: 2.1.1.150], and 4'-methoxy isoflavone 2'-hydroxylase [EC: 1.14.14.90 1.14.14.89] respectively. Flavanone liquiritigenin (7,4′-dihydroxyflavanone) is the precursor of Daidzein, Formononetin, and Glycitein; on the other hand, the precursor of Genistein and Biochanin-A is Naringenin (5,7,4′-dihydroxyflavanone) (Ko, 2014). By the presence of the enzyme 2-hydroxyisoflavanone synthase [EC: 1.14.14.87] Liquiritigenin or 4',7-Dihydroxyflavanone ($C_{15}H_{12}O_4$) gives rise to 2,7,4'-Trihydroxyisoflavanone ($C_{15}H_{12}O_5$) which in turn is converted into Daidzein ($C_{15}H_{10}O_4$) by the enzyme 2-hydroxyisoflavanone dehydratase [EC: 4.2.1.105] and converts Daidzein into Formononetin ($C_{16}H_{12}O_4$) by the activity of the enzyme isoflavone 4'-O-methyltransferase [EC:2.1.1.212 2.1.1.46] (**Fig. 4**).

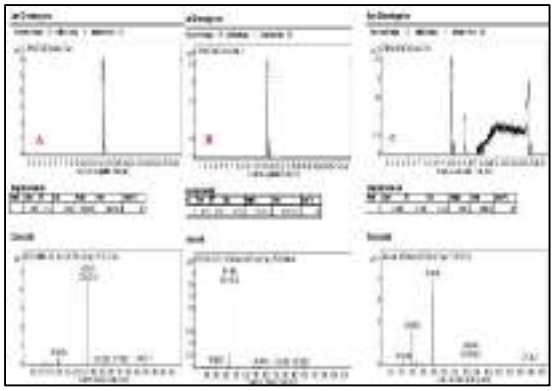

**Fig. 2** HR-LCMS of three major isoflavones namely (A) Biochanin-A, (B) Formononetin, and (C) Genistein found in mung bean sprouts.

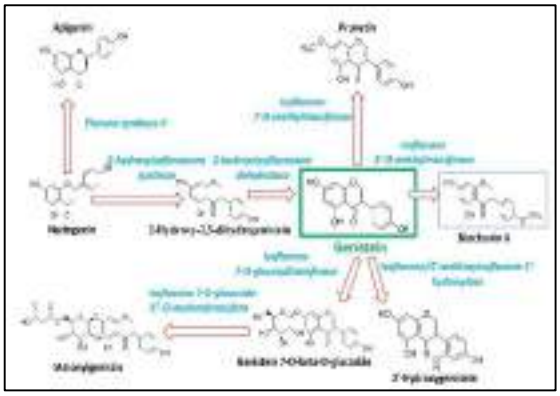

**Fig. 3** Derivation and glycosylation of Genistein and Biochanin-A through the KEGG pathway

***Antioxidant activity and oxidative stress management by SMP with chitosan and nano-chitosan***

As compared to untreated plants, SMP with nano-chitosan significantly reduced the amount of $H_2O_2$ in the study. Similar patterns were visible in MDA content. The levels of MDA and $H_2O_2$ were higher in untreated plants. However, compared to untreated (control) plants, primed plants were found to have higher flavonoid, ascorbic acid, and proline contents, which may indicate that oxidative damage caused by ROS has reduced the integrity and stability of the plasma membrane. In general, plants exposed to nano-chitosan showed an increase in antioxidant activities like catalase (EC: 1.11.1.6) and ascorbate peroxidase (EC: 1.11.1.11). Similar to this, treated plants had higher PPO and peroxidase (EC: 1.11.1.7) activities than untreated plants did. In contrast to plants treated with nano-chitosan, chitosan-treated plants had slightly higher values. When compared to untreated plants grown under salinity stress, SOD values in treated plants showed a significant decline. SOD is a major $O_2^-$ scavenger that catalyzes $O_2^-$ to $H_2O_2$ and $O_2$ (Apel and Hirt 2004). After that, POD, CAT, or ascorbate peroxidase can be used to remove the hazardous $H_2O_2$ (Foyer and Noctor 2005; Kusvuran et al. 2020). Ascorbic acid was used as a benchmark for the ABTS radical scavenging activity. Previous publications have described the use of ascorbic acid, a common water-soluble antioxidant found in foods, as a standard for the ABTS assay (Tang et al. 2010). Similar outcomes in the DPPH assay were attained. High levels of $H_2O_2$ in mung bean plants are a sign of excessive salt-induced ROS production and are caused by membrane lipid peroxidation as a result of salt-induced oxidative damage. These findings in various plants grown under salt stress are supported by numerous reports (Shabala and Munns 2012). The SMP-induced improvement over salinity stress in terms of its alleviation is demonstrated in the current study by lower levels of $H_2O_2$ and MDA measured in treated mung bean plants.

Proline levels were found to be higher in plants treated with nano-chitosan, which also scavenges ROS and stabilizes biomolecules and bio-membranes. In order to restore their water balance, stressed plants collect osmolytes in the interior of their cells. The increase in Proline content in response to salt stress suggests that mung bean plants can adapt to osmotic stress brought on by salt. The SMP treatment with nano chitosan improved this capacity for dealing with salt stress even more, as shown by the continued rise in Proline content. Proline is widely known for its semi-protective

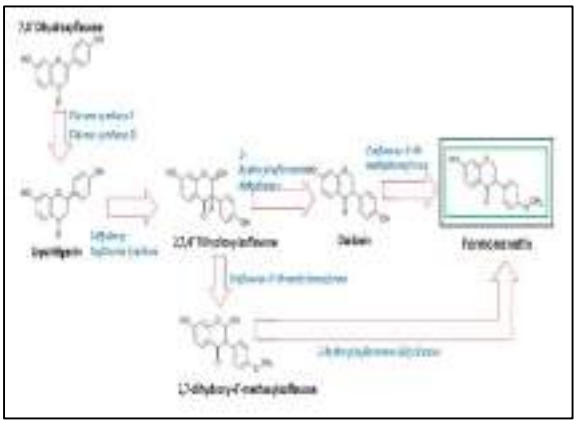

**Fig. 4** Biosynthesis of Formononetin from flavones through the KEGG pathway

property and hydroxyl radical scavenging activity, which stabilizes bio-membranes and biomolecules both structurally and functionally (Ahmad et al. 2010). By defending the photosynthetic apparatus, proline also improves photosynthesis. As a result, it helps plants survive and adapt to salt stress by acting as an energy storage material. The osmoregulation, ROS scavenging, and stabilization of membranes as well as macromolecules like protein and DNA improved after SMP treatment with nano chitosan under salt stress in mung bean plants. The current study found that SMP with nano chitosan increased the level of proline in mung bean plants under salt stress, which is consistent with a previous report on pro levels in cadmium-stressed mung bean (Nahar et al. 2016). The current study's findings thus suggest that SMP containing nano-chitosan can reduce oxidative damage and control the solidity of the plasma membrane system under salt stress.

***PCA biplots & Heat map analysis***

PCA, a multivariate statistical technique, is frequently used to keep as much information as possible while reducing the dimensionality, or the number of variables, of a large number of correlated variables. Thus, the multivariate data set undergoes a linear transformation into a set of uncorrelated variables that are organized in descendant form by the variance explained. The PCA biplot in this study illustrates the relationships between variables including MDA, proline content, ascorbic acid content, and all of the enzymological assays carried out (POD, APX, CAT, PPO, and SOD) (**Fig.** 5).

The first principal component (PC1) shapes 84.15% and thus most of the studied attributes confined around it. Except ABTS, DPPH and flavonoid all other attributes are on the negative axis of PC1. Antioxidant enzymatic activity correlated positively

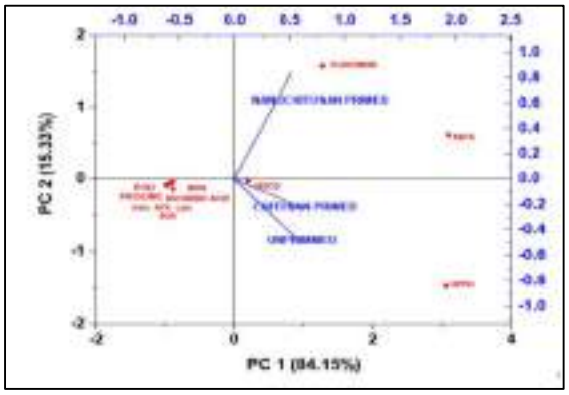


**Fig. 5** PCA biplot of different treatments and biochemical attributes studied showing antioxidant properties.

with stress indicators, suggesting a defensive response to stress induction. However, when looking at stress markers in terms of IC50, antioxidant activity was found to have a negative correlation. Thus, PCA clustering clearly shows that mung bean seeds primed with chitosan and nano-chitosan are prospective enough to enhance seed germination by removing pre-germination stress through activating defensive secondary metabolites.

On the other hand, the heat map is a data matrix that uses a color gradient to show the values in the cells. In the present investigation, the heat map (**Fig.** 6) between the chosen parameters and characteristics of mung bean seedlings in the current investigation provides a clear overview of the highest and lowest values in the matrix. Evidently, nano-chitosan primed seedlings clearly showed enhanced activities of APX, PPO, POD, CAT and SOD. Proline content and MDA content were also significantly improved after nano-chitosan priming in comparison to the unprimed and chitosan treated ones.

***Cytoscape analysis***

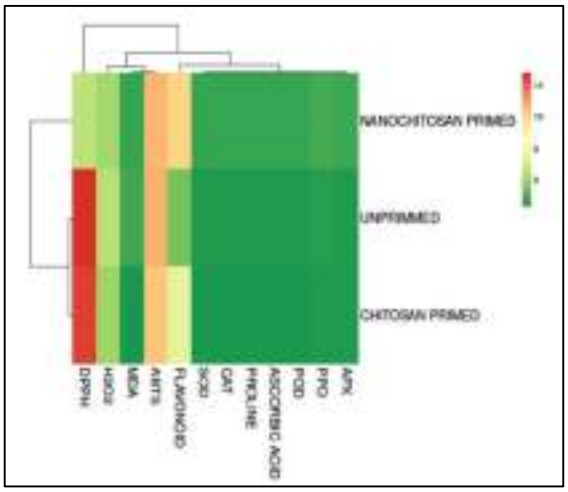


**Fig. 6** Heat map between the selected parameters and attributes of mung bean seedlings grown under salt stress conditions.

The relationship between the chosen isoflavones and the salt stress genes is shown in **Fig.** 7. It is discovered that certain genes, including SOS1, SOS2, SOS3, CBL1, CBL10, NHX1, AKT1, and VPS34, play a role in the plant body's response to salt stress (pathway ID GO: 0009651). Furthermore, potassium ion homeostasis is regulated by the genes SOS3, CBL3, and NHX1 (pathway ID GO: 0055075). Ion transporters, such as the $Na^+/H^+$

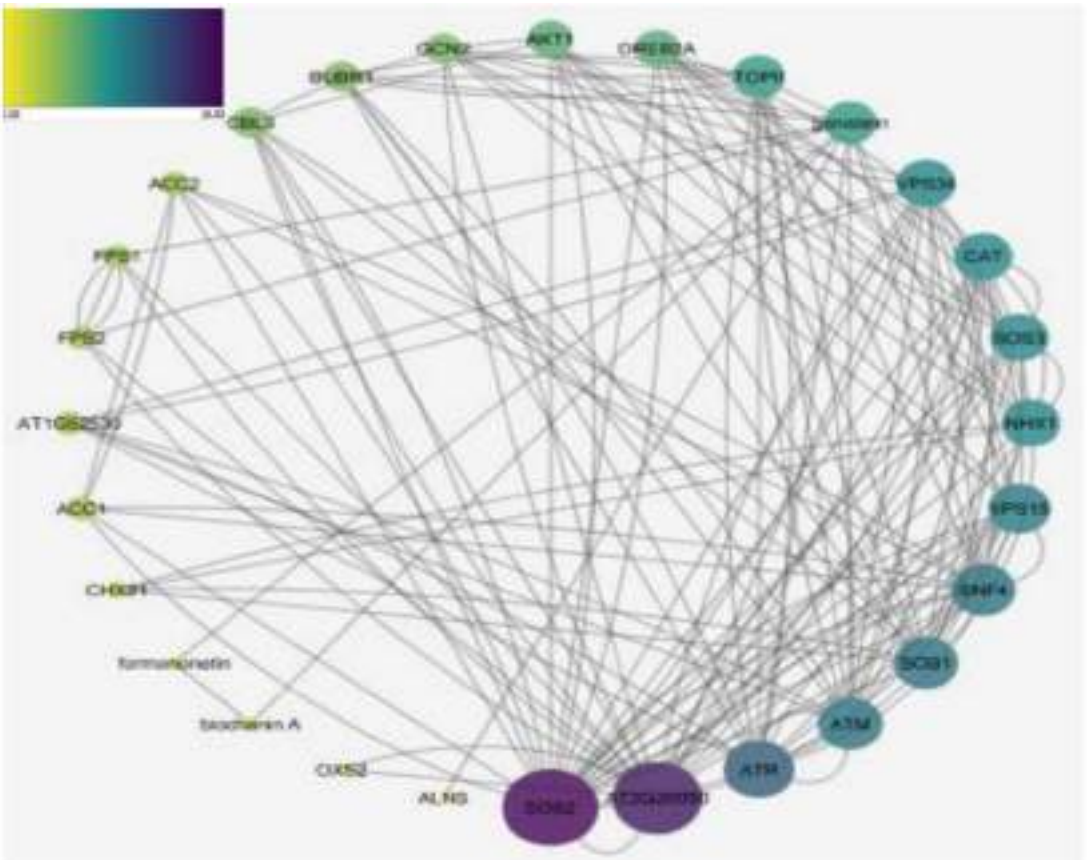


**Fig. 7** The correlation between the selected isoflavones and the salt stress genes is represented through Cytoscape analysis.

antiporter SALT OVERLY SENSITIVE 1 (SOS1) and the $K^+$ rectifier ARABIDOPSIS $K^+$ TRANSPORTER 1 (AKT1), correctly maintain a high $K^+/Na^+$ ratio in the cytoplasm, a phenomenon essential for salt tolerance (Chen et al. 2005; Feng et al. 2015). The electrochemical gradient created by transmembrane proton pumps, such as plasma membrane $H^+$-ATPase, vacuolar $H^+$-ATPase (V-ATPase), and vacuolar $H^+$-translocating inorganic pyrophosphatase (V-PPase), energizes these secondary transporters (Chen et al. 2010; Yang et al. 2010; Yuan et al. 2016). It is noteworthy that the plasma membrane $H^+$-ATPase is a critical site susceptible to various stresses, including salt stress, cold stress, heavy metal stress, and stress from active transport of the stressor across the plasma membrane (Martz et al. 2006; Shi et al. 2008; Janicka-Russak et al. 2012). In Arabidopsis thaliana, fatty acid biosynthesis (KEGG pathway ID 00061), fatty acid metabolism (KEGG pathway ID 01212), and pyruvate metabolism (KEGG pathway ID 00620) are all regulated by the genes ACC1, CAC1, and BCCP2. The genes BCCP2, CAC1, KIN11, ACC1, ACC2, and VPS34 are also involved in the cellular lipid metabolic process (pathway ID GO: 0044255) in Arabidopsis thaliana. Surprisingly, acetyl-CoA is also a factor in histone acetylation, giving peroxisomal FA oxidation control over nuclear epigenetic modification that may have an impact on a variety of cellular processes (Wang et al. 2019).

## Conclusions

In the present investigation, the seeds were only treated with nano-chitosan for 24 hours, but the triggering impact was seen for a few days. In this case, it could be said that the NPs are taken up by the surface of the seeds and slowly released over the course of a week to have their effect. This study looked at how SMP with nano-chitosan affected the antioxidant activities of mung bean sprouts, especially the isoflavones found in mung beans, and their relationships to genes for salinity tolerance. When used as a priming agent in SMP, nano-chitosan not only improved the ability of mung bean seedlings to withstand salinity but also enhanced

their antioxidant capacities in comparison to untreated seedlings, which is linked to the metabolic shifting of important isoflavones with potential therapeutic applications. The results of this study suggest that SMP with nano-chitosan may be a useful technique for producing functional foods with high metabolic contents and isoflavones, which would enhance the nutritional value and health benefits of mung bean sprouts.

**Acknowledgement**

The valuable contribution of Mr. Kushankur Sarkar to accomplish the Cytoscape study is sincerely acknowledged.

**Conflicts of interest**

There are no actual or potential conflicts of interest to declare.

**References**

Abbasi Khalaki M, Moameri M, Asgari Lajayer B, Astatkie T (2021) Influence of nano-priming on seed germination and plant growth of forage and medicinal plants. Plant growth regulation 93:13-28.

Aebi H (1984) Catalase in vitro. Method of Enzymology 105:121–126.

Ahmed B, Rizvi A, Zaidi A, Khan MS, Musarrat J (2019) Understanding the phyto-interaction of heavy metal oxide bulk and nanoparticles: evaluation of seed germination, growth, bioaccumulation, and metallothionein production. RSC advances 9(8):4210-4225.

Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S (2010) Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. Critical Reviews in Biotechnology 30:161-175.

An J, Hu P, Li F, Wu H, Shen Y, White JC, Tian X, Giraldo JP (2020) Emerging investigator series: molecular mechanisms of plant salinity stress tolerance improvement by seed priming with cerium oxide nanoparticles. Environmental Science: Nano 7(8):2214-2228.

Anusha JR, Fleming AT (2016) Synthesis and characterization of chitosan nano-aggregates from gladius of *Uroteuthis duvauceli*. International Journal of Biomaterials. https://doi.org/10.1155/2016/5379424

Apel K, Hirt H (2004) Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55:373-399.

Atanassova M, Georgieva S, Ivancheva K (2011) Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. Journal of Chemical Technology and Metallurgy 46(1):81-88.

Bates LS, Walderen RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39:205–207. https://doi.org/10.1007/BF00018060

Bellou S, Karali E, Bagli E, Al-Maharik N, Morbidelli L, Ziche M, Adlercreutz H, Murphy C, Fotsis T (2012) The isoflavone metabolite 6-methoxyequol inhibits angiogenesis and suppresses tumor growth. Molecular cancer 11(1):1-11.

Chacko BK, Chandler RT, D'Alessandro TL, Mundhekar A, Khoo NK, Botting N, Barnes S, Patel RP (2007) Anti-inflammatory effects of isoflavones are dependent on flow and human endothelial cell PPAR γ. The Journal of nutrition 137(2):351-356.

Chacko BK, Chandler RT, D'Alessandro TL, Mundhekar A, Khoo NK, Botting N, Barnes S, Patel RP (2007) Anti-inflammatory effects of isoflavones are dependent on flow and human endothelial cell PPAR γ. The Journal of nutrition 137(2):351-356.

Chen GX, Asada K (1989) Ascorbate peroxidase in tea leaves: Occurrence of two isoenzymes and the differences in their enzymatic and molecular properties. Plant and Cell Physiology 30: 987-998.

Chen Z, Newman I, Zhou M, Mendham N, Zhang G, Shabala S (2005) Screening plants for salt tolerance by measuring $K^+$ flux: a case study for barley. *Plant, Cell,* and *Environment* 28:1230–1246. https://doi.org/10.1111/j.1365-3040.2005.01364.x

Chen M, Song J, Wang BS (2010) NaCl increases the activity of the plasma membrane $H^+$-ATPase in C-3 halophyte *Suaeda salsa* callus. Acta Physiologiae Plantarum 32:27–36. https://doi.org/10.1007/s11738-009-0371-7

Conklin CM, Bechberger JF, MacFabe D, Guthrie N, Kurowska EM, Naus CC (2007) Genistein and quercetin increase connexin43 and suppress growth of breast cancer cells. Carcinogenesis 28(1):93-100.

Connolly A, O'Keeffe MB, Piggott CO, Nongonierma AB, FitzGerald RJ (2015) Generation and identification of angiotensin-converting enzyme (ACE) inhibitory peptides from a brewers' spent grain protein isolate. Food Chemistry 176:64-71.

Cornwell T, Cohick W, Raskin I (2004) Dietary phytoestrogens and health. Phytochemistry 65(8):995-1016.

Dakora FD, Phillips DA (1996) Diverse functions of isoflavonoids in legumes transcend anti-microbial definitions of phytoalexins. Physiological and Molecular Plant Pathology 49(1):1-20.

Davenport SB, Gallego SM, Benavides MP, Tomaro ML (2003) Behaviour of antioxidant defense system in the adaptive response to salt stress in *Helianthus annuus* L. cells. Plant Growth Regulation 40:81-88.

Dhayakaran RPA, Neethirajan S, Xue J, Shi J (2015) Characterization of antimicrobial efficacy of soy isoflavones against pathogenic biofilms. LWT-Food science and technology 63(2):859-865.

Dixon RA, Sumner LW (2003) Legume natural products: understanding and manipulating complex pathways for human and animal health. Plant Physiology 131(3):878-885.

do Espirito Santo Pereira A, Caixeta Oliveira H, Fernandes Fraceto L, Santaella C (2021) Nanotechnology potential in seed priming for sustainable agriculture. Nanomaterials 11(2):267.

Chandrasekaran U, Luo X, Wang Q, Shu K (2020) Are there unidentified factors involved in the germination of nanoprimed seeds? Frontiers in Plant Science 11:832.

Du M, Xie J, Gong B, Xu X, Tang W, Li X, Li C, Xie M (2018) Extraction, physicochemical characteristics and functional properties of Mung bean protein. Food Hydrocolloids 76:131-140; DOI: 10.1016/J.FOODHYD.2017.01.003

Dubin SB, Lunacek JH, Benedek GB (1967) Observation of the spectrum of light scattered by solutions of biological macromolecules. Proceedings of the National Academy of Sciences of the United States of America 57:1164.

Erba D, Casiraghi MC, Martinez-Conesa C, Goi G, Massaccesi L (2012) Isoflavone supplementation reduces DNA oxidative damage and increases O-β-N-acetyl-D-glucosaminidase activity in healthy women. Nutrition research 32(4):233-240.

Esfandiari E, Shekari F, Shekari F, Esfandiari M (2007) The effect of salt stress on antioxidant enzymes, activity and lipid peroxidation on the wheat seedling. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. https://doi.org/10.15835/nbha.35.1.251

Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17:1866–1875. https://doi.org/10.1105/tpc.105.033589

Gan RY, Lui WY, Wu K, Chan CL, Dai SH, Sui ZQ, Corke H (2017) Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. Trends in Food Science and Technology 59:1-14.

Gao Y, Yao Y, Zhu Y, Ren G (2015) Isoflavone Content and Composition in Chickpea (*Cicer arietinum* L.) Sprouts Germinated under Different Conditions. Journal of agricultural and food chemistry 63(10): 2701. https://doi.org/10.1021/jf5057524

Guha T, Ravikumar KVG, Mukherjee A, Mukherjee A, Kundu R (2018) Nanopriming with zero valent iron (nZVI) enhances germination and growth in aromatic rice cultivar (*Oryza sativa* cv. *Gobindabhog* L.). Plant Physiology and Biochemistry 127:403-413.

Jefferson WN, Williams CJ (2011) Circulating levels of genistein in the neonate, apart from dose and route, predict future adverse female reproductive outcomes. Reproductive toxicology 31(3):272-279.

Jia T, An J, Liu Z, Yu B, Chen J (2017) Salt stress-induced soybean GmIFS1 expression and isoflavone accumulation and salt tolerance in transgenic soybean cotyledon hairy roots and tobacco. Plant Cell Tissue and Organ Culture 128(2):1-9; DOI: 10.1007/s11240-016-1124-0

Joshi A, Kaur S, Dharamvir K, Nayyar H, Verma G (2018) Multi-walled carbon nanotubes applied through seed-priming influence early germination, root hair, growth and yield of bread wheat (*Triticum aestivum* L.) Journal of the Science of Food and Agriculture 98(8):3148-3160.

Keesey J (1987) Biochemica Information, Boehringer Mannheim Biochemicals, Indianapolis, IN, edn. 1:58.

Ko KP (2014) Isoflavones: chemistry, analysis, functions, and effects on health and cancer. Asian Pacific Journal of Cancer Prevention 15(17):7001-7010.

Kusvuran S, Bayat R, Ustun AS, Ellialtioglu SS (2020) Exogenous proline improves osmoregulation, physiological and biochemical responses of eggplant under salt stress. Fresenius Environmental Bulletin 29:152-161.

Lai F, Wen Q, Li L, Wu H, Li X (2010) Antioxidant activities of water-soluble polysaccharide extracted from mung bean (*Vigna radiata* L.) hull with ultrasonic-assisted treatment. Carbohydrate Polymers 81(2):323-329.

Li H, Cao D, Yi J, Cao J, Jiang W (2012) Identification of the flavonoids in mung bean (*Phaseolus radiatus* L.) soup and their antioxidant activities. Food Chemistry 135(4):2942-2946.

Liu Y, Hassan S, Kidd BN, Garg G, Mathesius U, Singh, KB, Anderson JP (2017) Ethylene signaling is important for isoflavonoid-mediated resistance to *Rhizoctonia solani* in roots of *Medicago truncatula*. Molecular plant-microbe interactions 30(9):691-700.

Loreto F, Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiology 127:1781-1787.

Nahar K, Hasanuzzaman M, Alam MM, Rahman A, Suzuki T, Fujita M (2016) Polyamine and nitric oxide crosstalk: antagonistic effects on cadmium toxicity in mung bean plants through upregulating the metal detoxification, antioxidant defense, and methylglyoxal detoxification systems. Ecotoxicology and Environmental Safety 126:245–255. https://doi.org/10.1016/j.ecoenv.2015.12.026

Nishaa S, Vishnupriya M, Sasikumar JM, Hephzibah PC, Gopalakrishnan VK (2012) antioxidant activity of ethanolic extract of *Maranta arundinacea* L. tuberous rhizomes. Asian Journal of Pharmaceutical and Clinical Research 5(4): 85-88; ISSN - 0974-2441

Omaye ST, Turnbull JD, Sauberilich HE (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods in Enzymology 62:03-11.

Rajeshwari K, Latha S, Gomathi T, Sangeetha K, Sudha PN (2016) Preparation and Characterisation study of Nanochitosan (NCS) and polyvinyl alcohol (PVA) binary blends with glutaraldehyde as a crosslinking agent. Scholars Research Library, *Der Pharmacia Lettre* 8(19):485-495

Randhir R, Shetty K (2007) Mung beans processed by solid-state bioconversion improves phenolic content and functionality relevant for diabetes and ulcer management. Innovative Food Science and Emerging Technologies 8(2):197-204.

Rietjens IM, Sotoca AM, Vervoort J, Louisse J (2013) Mechanisms underlying the dualistic mode of action of major soy isoflavones in relation to cell proliferation and cancer risks. Molecular nutrition and food research 57(1):100-113.

Rípodas C, Dalla Via V, Aguilar OM, Zanetti ME, Blanco FA (2013) Knock-down of a member of the isoflavone reductase gene family impairs plant growth and nodulation in *Phaseolus vulgaris*. Plant physiology and biochemistry 68:81-89.

Rodríguez-Roque MJ, Rojas-Graü MA, Elez-Martínez P, Martín-Belloso O (2013) Soymilk phenolic compounds, isoflavones, and antioxidant activity as affected by in vitro gastrointestinal digestion. Food Chemistry 136(1):206-212.

Sen SK, Mandal P (2018) Application of solid matrix priming to ameliorate salinity stress in mung bean ('*Vigna radiata*'). Australian Journal of Crop Science 12(3):458-466.

Harman GE, Taylor AG (1988) Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. Phytopathology 78(5):520-525.

Sen SK, Jha S, Mandal P (2016) Evaluation of Phytotoxicity of Mung Bean genotypes against Salinity Stress. International Journal of Scientific and Research Publications 6(12):333-340.

Shabala S, Munns R (2012) Salinity Stress: Physiological Constraints and Adaptive Mechanisms. In: Shabala S (ed) Plant Stress Physiology. CAB International, Oxford, pp 59–93. https://doi.org/10.1079/9781845939953.0059

Shende S, Patel S, Arora S, Sharma V (2014) Oxidative stability of ghee incorporated with clove extracts and BHA at elevated temperatures. *International Journal* of *Food Properties* 17:1599–1611. https://doi.org/10.1080/10942912.2012.752382

Sidduraju P, Mohan P, Becker K (2002) Studies on the antioxidant activity of Indian Laburnum Cassia fistula L: A preliminary assessment of crude extracts from stem bark, leaves, flowers, and fruit pulp. Food Chemistry 79:61-7.

Souček J, Škvor J, Poučková P, Matoušek J, Slavík T, Matoušek J (2006) Mung bean sprout (*Phaseolus aureus*) nuclease and its biological and antitumor effects. Neoplasma 53(5):402-409.

Sirotkin AV, Harrath AH (2014) Phytoestrogens and their effects. European journal of pharmacology 741:230-236.

Sudha PN, Vinodhini PA, Sangeetha K, Latha S, Gomathi T (2014) Fabrication of cellulose acetatechitosan-polyethylene glycol ultrafiltration membrane for chromium removal, Der Pharmacia Lettre 6(1):37-46.

Swigonska S, Amarowicz R, Król A, Mostek A, Badowiec A, Weidner S (2014) Influence of abiotic stress during soybean germination followed by recovery on the phenolic compounds of radicles and their antioxidant capacity. Acta Societatis Botanicorum Poloniae 83:209-218.

Tang X, He Z, Day Y, Xiong YL, Xie M, Chen J (2010) Peptide Fractionation and Free Radical Scavenging Activity of Zein Hydrolysate. Journal of Agricultural and Food Chemistry 58:587-593.

Wang HJ, Murphy PA (1994) Isoflavone content in commercial soybean foods. *Journal of Agricultural* and *Food Chemistry* 42:1666–1673. https://doi.org/10.1021/jf00044a016

Wang L, Wang C, Liu X, Cheng J, Li S (2019) Peroxisomal b-oxidation regulates histone acetylation and DNA methylation in *Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America. 116:10576–10585. https://doi.org/10.1073/pnas.1904143116

Yao Y, Yang X, Tian J, Liu C, Cheng X, Ren G (2013) Antioxidant and antidiabetic activities of black mung bean (*Vigna radiata* L.). Journal of Agricultural and Food Chemistry 61(34):8104-8109.

Ye Y, Cota-Ruiz K, Hernandez-Viezcas JA, Valdes C, Medina-Velo IA, Turley RS, Peralta-Videa JR, Gardea-Torresdey JL (2020) Manganese nanoparticles control salinity-modulated molecular responses in *Capsicum annuum* L. through priming: A sustainable approach for agriculture. ACS Sustainable Chemistry & Engineering 8(3):1427-1436.