Journal of Applied and Natural Science
15(2), 582 - 593 (2023)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Effects of foliar applications of ascobin on seed protein accumulation in soybean (*Glycine max* (L.) Merrill) JS 1215 grown under cadmium stress

Amit
Department of Botany, Kurukshetra University, Kurukshetra - 136119 (Haryana), India
Yogesh Kumar*
Department of Botany, Kurukshetra University, Kurukshetra - 136119 (Haryana), India

*Corresponding author. E-mail: ykskuk11@gmail.com

Article Info
https://doi.org/10.31018/jans.v15i2.4387
Received: January 15, 2023
Revised: May 9, 2023
Accepted: May 15, 2023

How to Cite
Amit and Kumar, Y. (2023). Effects of foliar applications of ascobin on seed protein accumulation in soybean (*Glycine max* (L.) Merrill) JS 1215 grown under cadmium stress. *Journal of Applied and Natural Science*, 15(2), 582 - 593. https://doi.org/10.31018/jans.v15i2.4387

Abstract
Different natural and anthropogenic activities have contributed to the prevalence of various environmental abiotic stressors that negatively affect agricultural yields worldwide. Also, more adverse impacts can be seen in legumes due to their susceptibility to abiotic stresses compared to cereals. The present study sought to understand cadmium's impact and its alleviation using ascobin on several seed protein properties of soybean (*Glycine max*). The total seed protein content, estimated using the semi-micro Kjeldahl method, was 36.25% without ascobin (control) at the highest cadmium (Cd) concentration, i.e., 30 mg per kg of soil. The protein content was restored to the highest level (43.75%) with 500 mg/L of ascobin at 10 mg Cd/kg of soil compared to the control without Cd. There was a noticeable decrease in total seed protein content in all sets under control conditions (i.e., without ascobin spray). Also, a negative correlation was found between increasing Cd concentration and the amount of free amino acids, quantified using Lee and Takahashi's protocol in the seed proteins. The electrophoretic analysis using Gelanalyzer on SDS gels, as per Laemmli's formulation, revealed that the 7S-Conglycinin protein subfraction was more affected than the 11S-Glycinin subfraction. The analysis revealed how Cd toxicity in soybean plants led to decreased seed protein content and altered proportion of two globulin sub-fractions (glycinin and β-conglycinin). Additionally, it affected the free amino acid content, potentially determining the seeds' nutritional value. However, foliar application of ascobin helped the plants to mitigate these Cd -induced changes and restore the seed quality.

Keywords: Ascobin, Cadmium, SDS-gel electrophoresis, Seed proteins, Soybean

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is India's most important Kharif crop among the oil and protein-producing legumes. It is a short-seasoned leguminous crop that thrives in hot weather with moderate to heavy rainfall (Singh, 2010). It is also needed for various industrial goods, such as cream, vegetable ghee, milk, cakes, paints, paints and coatings, gum, and glue, which give the crop a market (Riaz, 2005). Besides protein and oil, soybeans also contain isoflavones, non-nutritive compounds with health-promoting properties (Leitzmann, (2016). However, a number of abiotic elements, such as high salt concentration in soil, high or low water stress, heavy metals, etc., limit any plant crop's yield and quality, which is also true for soybean. Even though soybean has evolved to grow well in various climates, these abiotic factors considerably influence their growth, development, and total productivity (Tewari and Arora, 2016; Feng *et al.*, 2020).

The foliar spray of micro and macro-nutrients improves the plant's vegetative growth and seed biochemical composition. Foliar nutrients are supplied when demand is very high and a quick response is needed, like after heavy rain (Ali *et al.*, 2013). Numerous studies have demonstrated the positive effects of foliar fertilization in various plants, including lettuce, wheat, soybean, and many others (Fageria *et al.*, 2019). In this regard, antioxidants have synergistic effects on the development and production of various plant species. Antioxidants are natural and harmless chemicals that protect cells against oxidation, which can form free radicals. Therefore, the antioxidant foliar spray makes it possible for plants to recover from the effects of envi-

ronmental stress and produce their best (Kuchlan *et al.*, 2017) and (Teixeira *et al.*, 2017).

The ascobinas foliar fertilizer contains ascorbic and citric acids (2:1) and has been shown to have a stimulatory effect on growth and active phytoconstituents in many plants, including the maize, wheat (El-Maddah *et al.*, 2012), common rue (*Ruta graveolens*) (El-Sherbeny *et al.*, 2007), rice (Tahjib-Ul-Arif *et al.*, 2021), and cowpea (Abdelgawad, 2014). Sheteawi (2007) claimed ascobin spray could be used in soybeans as a growth regulator or antioxidant to enhance soybean growth, yield, and nutrient utilization at moderate salinity levels. According to Allahveran *et al.* (2018), ascobin, combined with jasmonic acid, enhanced soybean tolerance to salinity by boosting the deposition of non-toxic metabolites (sugars, amino acids, proline, and proteins). Also, it was found that the addition of ascorbic and citric acids has an auxinic activity and a synergistic effect on increasing fruit retention. Furthermore, ascobin enhanced many vital growth parameters and antioxidant enzyme activity under abiotic stress while reducing H_2O_2 and malondialdehyde (MDA) levels in wheat (Dadrwal *et al.*, 2018).

However, there needs to be more information on the effect of ascobin foliar applications on soybean total seed storage proteins and its major seed protein fraction, i.e., 7S and 11S globulins, particularly under heavy metals such as Cd. Thus, the present study sought to examine the function of ascobin applications in counteracting the detrimental effects of Cd on the seed storage proteins of soybean *Glycine max L.* (JS 1215) plants, as well as the importance of ascobin in increasing the resilience of soybean plants to Cd toxicity.

MATERIALS AND METHODS

To study the effects of foliar application with ascobin, soybean seeds (JS 1215 variety) collected from National Seeds Corporation Ltd. (NSC) in Rajasthan, India. Uniform-sized healthy seeds were selected manually and washed three times with lukewarm water just before sowing. The experiment was conducted in the first week of June in the experimental plots of the Department of Botany, Kurukshetra University, Kurukshetra. The soil-filled pots were separated into five sets, each with five replicates, including one set as the control (without Cd but with water spray), a second set with Cd and water spray, and the rest three with heavy metal ascobin treatments. A crop thinning was carried out to maintain a density of five plants per container. Considering the permissible limit of Cd (3mg/kg) (Kinuthia *et al.*, 2020), in the soil and the tolerance range of legumes to this metal, in each set, three concentration levels of the Cd (as $CdCl_2$. H_2O) 10mg/kg, 20mg/kg, and 30 mg/kg of soil were supplied in solution form after seedling establishment. Ascobin having 0.1 (v/v) Tween -20 as a surfactant, in three different concentrations, i.e., 150mg/L, 300mg/L and 500mg/L of water, was sprayed on plants throughout their vegetative growth starting after one week of Cd supply. There was a total of four sprays of ascobin with an interval of 15 days to ameliorate the negative effect of Cd. In contrast, water with Tween-20 served as the control, as represented in Table 2. Additionally, plants were irrigated from time to time as required. Mature seeds were collected during the second week of October, 2021 dried, and then crushed into seed meals for the proposed study.

Seed protein isolation and characterization

The seeds collected at maturity were ground and defatted using hexane (1g seed meal/10 ml hexane) at 4ºC for two hours. The defatted seed meal was used for further studies on seed protein characteristics such as total seed protein content, the proportion of 11S and 7S sub-fractions, polypeptide pattern SDS-gel electrophoresis, etc.

Protein estimation

The semi-micro Kjeldahl method was used to determine the total seed protein content (Vogel, 1960). The seed meal was digested with sulfuric acid in the presence of copper sulphate, selenium dioxide, and potassium dichromate as a catalytic mixture. For the nitrogen determination, the digest was heated with 40% NaOH in Markham's distillation assembly. The ammonia produced was fixed in boric acid that was further volumetrically titrated with N/40 HCl to get the nitrogen content. Multiplying the nitrogen with 6.25 yielded the total seed protein content. The free amino acids were quantified according to Lee and Takahashi (1966). The seed proteins were isolated and sorted into 7S and 11S globulins using the method described by Thanh and Shibasaki (1976). Bradford (1976) technique was used to calculate the protein content of the samples relative to Bovine serum albumin (BSA) as the standard.

Preparation of total seed protein extract

20 mg of defatted seed meal was extracted in 200 µl of 0.025M Tris-HCL (pH 6.8) containing 2% sodium dodecyl sulphate (SDS). Followed by heating in a water bath at 80°C for 40 minutes while constantly stirring with a vortex. After that, it was centrifuged at 12,000g using a Remi microfuge, and 10% (v/v) glycerol was added to the separated supernatant. To prepare the extract for electrophoresis under reducing conditions, 2% 2-mercaptoethanol was added. Before being loaded onto the gels, the samples were heated for 10 minutes at 100 degrees Celsius (Singh and Matta, 2008).

SDS-polyacrylamide gel electrophoresis

Following the discontinuous system described by Davis

(1964) and Ornstein (1964) and the formulation of Laemmli (1970), the polypeptide patterns of seed protein were analyzed electrophoretically on 14% polyacrylamide SDS gels under reducing conditions. 1.5 millimetres thick perspex spacers and 24x21 centimetres glass plates were used to prepare the SDS-gel. During electrophoresis in the stacking gel with a pH of 6.8, a current of 18 mA was run, and this current was raised to 32 mA in the separation gel having pH of 8.8. Coomassie Brilliant Blue R-250 was used to stain the protein bands, and standard marker proteins were also run on the gel to determine their molecular weights.

Densitometry of polypeptides

A calibration curve was prepared by plotting the relative mobility of markers against the log of their standard molecular weights and figuring out the molecular weights and band intensity peak value of polypeptides in total seed protein extract (Weber and Osborn, 1969). GelAnalyser, downloaded from www.gelanalyzer.com, was used for densitometric scanning of the polypeptides. It analyses gel pictures captured by a scanner, camera, or digital camera. It allows visual control of band identification and comparison analysis taking into account the relative electrophoretic mobility (Rf) of SDS -gel bands. It also enables the evaluation of poorly resolved bands and the elimination of image traces. The GelAnalyzer program calculates normalized coordinates of bands, compares their spectra to reveal similarities or differences in their components, and provides quantitative evaluation and analysis of SDS-gel bands. For each SDS-gel lane, the intensity or raw volume of the total protein represented the relative concentration of each protein or polypeptide. In the present calculations, all raw volumes were divided by one thousand for simplicity of data and graphs. For our calculation, gel images were obtained using 'ImageQuant 100' image-capturing device and run through GelAnalyzer.

RESULTS AND DISCUSSION

Seed protein content

Soybean (*Glycine max* L.) plants responded differently to various concentrations of Cd (10, 20 and 30mg per kg of soil) and ascobin. The study observed positive outcomes of ascobin spray in Cd-stressed plants regarding total seed protein content. The protein content in set 1 C-0 (without any kind of treatment) was noted to be 41.88%. As Cd concentration was increased (C-1 to C-3), the seed protein decreased from 38.7% to 36.25%. However, ascobin alleviation was much more at Cd concentration level 2, i.e., 20mg/kg (6 C-2), where the total seed protein increased to 42.5% (9 T-9) from 36.87% (control 6C-2), which was the highest in present study represented in Fig.1. Without ascobin spray, the seed protein content reduced as the Cd stress concentration was increased. It increased from 38.7% (control 2 C-1) to 41.25 (5 T-3) as the ascobin concentration was increased from 150mg/L to 500mg/L at 10mg/kg Cd concentration. Similarly, at Cd 20mg/kg (control 6 C-2), the total seed protein increased from 36.87% to 42.5% (9 T-3) with a change in ascobin concentration from 150mg/L to 500mg/L. At the third concentration level of Cd, i.e., 30mg/kg, the total seed proteins reached 40.25% (12 T-2) from 36.25% (control 10 C-3) at 300mg/L concentration level of ascobin spray (Fig. 1).

The present analysis revealed that cadmium at higher concentrations in soil altered the seed protein properties in soybean. Similar to the present study, a de-

Table 1. Showing SDS-gel lanes loaded with soybean JS1215 seed protein with different combinations of cadmium and three levels of ascobin. Also, represents the total nitrogen % and two subfractions %

Lane	Cadmium treatment	Ascobin	Nitrogen Content (%)	7S β-conglycinin (%)	11S Glycinin (%)
1 C-0	NA	Only water spray	6.7	28.2	25.6
2 C1	Cd level 1	Only water spray	6.2	20.5	21.1
3 T1	Cd level 1	level 1	6.4	25.7	24.3
4 T2	Cd level 1	level 2	6.4	26.8	24.7
5 T3	Cd level 1	level 3	6.6	27.8	25.1
6 C2	Cd level 2	Only water spray	5.9	19.1	20.9
7 T1	Cd level 2	level 1	6.6	24.5	22.1
8 T2	Cd level 2	level 2	6.6	27.6	23.1
9 T3	Cd level 2	level 3	6.8	30.2	22.4
10 C3	Cd level 3	Only water spray	5.8	19.5	20.8
11 T1	Cd level 3	level 1	6.1	26.5	22.6
12 T2	Cd level 3	level 2	6.5	25.3	23.2
13 T3	Cd level 3	level 3	6.4	28.8	24.5

Table 2. Showing the experimental plan performed to find out the effect of ascobin on soybean grown under various Cd concentrations

Set	Cadmium concentration	Control	Treatment 1	Treatment 2	Treatment 3
1 C-0	Nil	Only water spray	NA	NA	NA
1.	Cd 10 mg/kg	Only water spray (C-1)	Ascobin 150mg/ltr	Ascobin 300mg/ltr	Ascobin 500mg/ltr
2.	Cd 20 mg/kg	Only water spray (C-2)	Ascobin 150mg/ltr	Ascobin 300mg/ltr	Ascobin 500mg/ltr
3.	Cd 30 mg/kg	Only water spray (C-3)	Ascobin 150mg/ltr	Ascobin 300mg/ltr	Ascobin 500mg/ltr

crease in the seed protein content was reported in soybean when it was subjected to Cd stress (Hossain *et al.*, 2012). A decreased rate of protein synthesis or increased protein breakdown may be responsible for the reduction in total seed protein content (Mao *et al.*, 2018). According to Farooq *et al.* (2018), higher concentrations of heavy metals reduce the overall seed protein content in legumes. In addition, albumins and globulins, the seed protein fractions, have been reported to decrease under Cd supply in chickpeas (Ghosh *et al.*, 2022). These findings also validate the conclusions of Chaoui and El Ferjani (2013) and Jaouani *et al.* (2018) in lentils and peas, respectively.

It is well understood that the accumulation of proteins in the developing seeds results from the interplay of various genetic and environmental factors. Heavy metals can negatively impact plant functions in several ways, including by altering the structure of enzymes and proteins by interacting with their key components. It has also been observed that higher concentrations of heavy metals have a declining influence on the overall amount of seed proteins (Wani *et al.*, 2007). A reduction in nitrogenase activity has been reported in soybean on exposure to Cd (Balestrasse *et al.*, 2003). Under such abiotic stress, reactive oxygen species (ROS) are generated to a large level which is detrimental to the cellular structures of plants. ROS also oxidise proteins and lipids and negatively impact DNA as reported by Lin and Aarts (2012).

Ascobin exogenous application has enhanced yield and yield characteristics in treated plants and strongly counterbalanced the detrimental effect of abiotic stress in agricultural crops (Khan and Ashraf, 2008, Khan *et al.*, 2011). When ascobin was supplied to cowpea, the production increased by 6 to 9% (Abdelgawad, 2014). Abdelgawad *et al.* (2018) reported that foliar spray with ascobin improved the leaf content of chlorophyll content, nitrogen, phosphorus, total phenol, and total amino acids in head lettuce.

When citric acid, a component of ascobin is applied exogenously to mustard leaves (*Brassica juncea*) enhances the enzymatic antioxidant response by increasing superoxide dismutase, peroxidase and catalase activities, therefore mitigate the deleterious effects of Cd. Compared to mustard plants under Cd stress, citric acid-treated plants have increased their chlorophyll content and stomatal conductance, leading to a 14% increase in CO_2 availability and a 42% improvement in photosynthesis 12% increase in growth (Faraz *et al.*, 2020).Citric acid foliar application improves the total carbohydrate and seed protein content in wheat (Sadak and Orabi, 2015). Ascorbic acid another component of ascobin has also been reported to mitigate the harmful effects of Cd on wheat by enhancing photosynthetic efficiency, antioxidant enzyme activities, and soluble protein content. It also reduced Cd absorption, thus ameliorating the detrimental effects of Cd (Zhou *et al.*, 2022). Ascorbic acid decreased Cd absorption in maize, reducing oxidative damage via up-regulating antioxidant enzymes and non-enzymatic antioxidants and substantially reducing ROS-induced lipid peroxidation (Zhang *et al.*, 2019).

Seed protein sub-fractions

Under varying concentration levels of Cd and ascobin, the content of β-conglycinin fraction ranged from 19.1 to 30.2%, while the glycinin varied between 20.8 and 25.1% (Fig. 2). In set 1 C-0, the value of β-conglycinin and glycinin were noted as 28.2 and 25.6, respectively. In the set with 10mg per kg soil of Cd without ascobin the β-conglycinin in control was observed to be 20.5 (2 C-1) and with three different ascobin treatments, it increased to 25.7 (3T-1), 26.8(4 T-2) and 27.8 (5 T-3). Glycinin in the control set was noted to be 21.1 (2 C-1) and with ascobin application, it reached 24.3 (3 T-1), 24.7 (4 T-2) and 25.1 (5 T-3).

In second set with a Cd concentration of 20mg and without ascobin, the β-conglycinin was found to be19.1 (6 C-2), and with ascobin treatment, it increased to 24.5 (7 T-1), 27.6(8 T-2) and 30.2 (9 T-3). In this set, the glycinin was noted as 20.9 (6 C-2) in control and in ascobin treated plants, it was recorded to be 22.1 (7 T-1), 23.1 (8T-2) and 22.4 (9 T-3). In the set with 30 mg of Cd, the β-conglycinin and glycinin were noted to be 19.5 and 20.8 (10 C-3), respectively. With ascobin treatment, both fractions were also seen to increase shown in Fig. 2.

We studied the effect of Cd on soybean major fractions, i.e., the 7S (β-conglycinin) and 11S (glycinin) fractions and it was found that the total yield of glycinin and β-conglycinin showed differences with change in concentration of ascobin. According to Krishnan (2002), β-

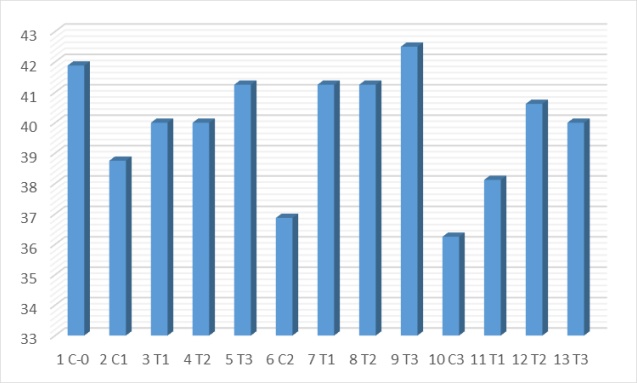

Fig. 1. *Seed protein content (%) under different concentrations of Cadmium and ascobin applications (For abbreviations*

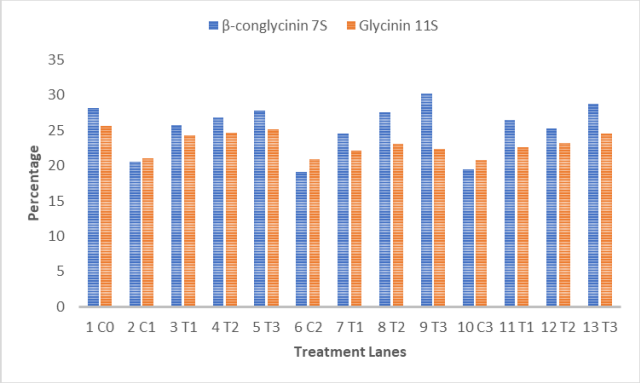

Fig. 2. *Soybean major seed protein fractions in according to different Cd and ascobin treatments*

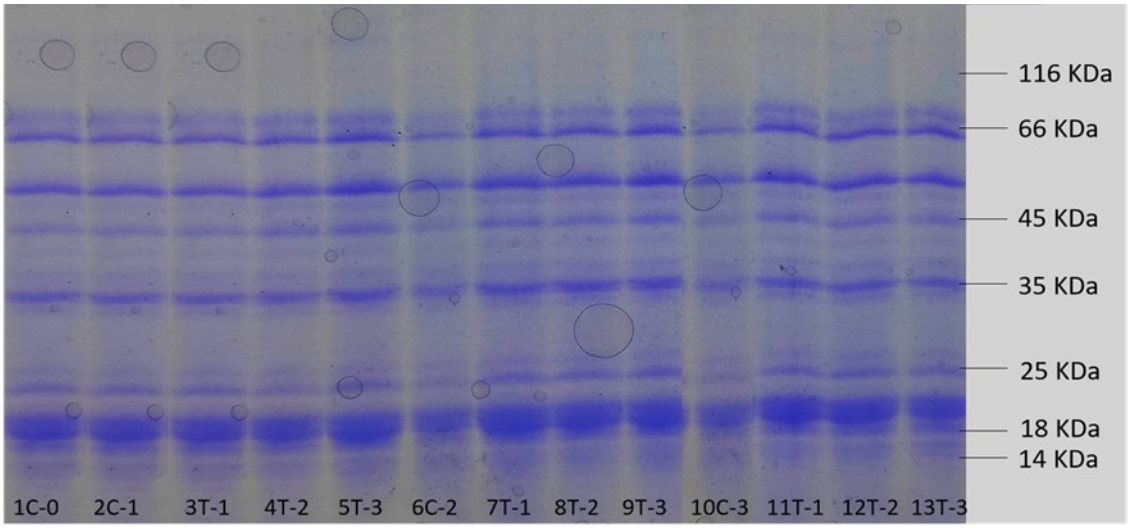

Fig. 3. *Showing SDS-gel of soybean seed protein and major fractions from soybean plants grown at various Cd and ascobin concentrations*

conglycinin is a key storage protein in soybean that accumulates differently throughout seed development. In order to provide energy for the quickly developing embryo and seedling, β-conglycinin is degraded during seed germination and early seed development. In another study on soybean, discovered a decreased accumulation of β-conglycinin was recorded during seed development in heavy metals polluted sites (Klubicova *et al.*, 2012). Also, they studied the abundance profiles of glycinin seed storage proteins varied throughout soybean development under stressful conditions. However, the difference was not statistically significant. Seed growth and development are negatively impacted by abiotic stresses such as drought, salt, heavy metal toxicity, heat stress, etc., which in turn affect biomass, yield, total seed protein content and its major subfractions (Juhász *et al.*, 2018, Luo *et al.*, 2018, and Nagy-Réder *et al.*, 2022).

Free Amino Acids

In addition, to being a good source of protein, soybean seeds also provide essential fatty acids and other nutrients. The present study found that the proportion of total amino acids fluctuated under Cd and ascobin supply. The content of free amino acids in set 1 C-0, was found to be 0.68%. The content of free amino acids was found to be 0.55%, 0.44% and 0.36% at 10mg/kg, 20mg/kg and 30mg/kg of Cd, respectively. Conversely, an increase in the percentage of free amino acids was observed when plants were sprayed with ascobin (Fig. 13). Under Cd stress, the content of free amino acids was noted to be 0.67%, 0.65% and 0.59% at Cd level 1, 2 and 3, respectively, with 500mg/l of ascobin application.

Some amino acids, such as proline, accumulate in plants which may help reduce the harmful effects of heavy metal accumulation (Mehta and Gaur, 1999). It has been shown that the levels of the amino acids tryptophan, cysteine, and methionine in protein fractions of chickpea seeds are reduced when exposed to Cd and Pb, as reported by Ghosh *et al.* (2022). Gianazza *et al.* (2007) observed that in *Lepidium sativum*, Cd was more damaging than Pb in terms of its effect on amino acid content in the seedling. ROS are produced in reac-

tion to heavy metals, which is likely to cause DNA damage, impaired DNA repair pathways, alter membrane function, nutrition absorption, and protein tertiary structure, protein functions etc. (Tamás *et al*., 2014). As a result of these alterations, the biosynthesis of amino acids is reduced under heavy metal stress (Garcia *et al*., 2006).

Polypeptide patterns on SDS-gels

The studies on the alterations in the accumulation and mobilization of seed proteins under abiotic stresses have been possible because of different electrophoretic techniques, particularly SDS-PAGE. Using this method, the present study demonstrated the substantial intensity variations in the polypeptide patterns of seed proteins of soybean plants exposed to Cd and ascobin. Under different concentration levels of Cd, along with and without ascobin, qualitative and quantitative changes were noticed in the polypeptide patterns on SDS gels. Intensity variations were seen in both β-conglycinin and glycinin polypeptides which were responsible for the differences in total seed proteins that can also be noticed in various lanes on the gel. As in earlier studies, the two sub-fractions in soybean seed proteins can be distinguished by their molecular weights (Liu *et al*., 2007). These subfractions were 7S β-conglycinin and 11S glycinin, with molecular weight ranges of 50kDa -75kDa and 20kDa - 38kDa respectively. In present study, the 7S β-conglycinin subfraction polypeptides were lightly stained, with relatively low intensity at higher Cd concentrations. These polypeptides exhibited higher intensity after ascobin treatment. However, the intensity differences in 11S glycinin subfractions with Cd and ascobin treatment were not as apparent as in the 7S subfraction. Thus, the 7S β-conglycinin subfraction seem to be more prone and sensitive to Cd stress than 11S glycinin as shown in Fig. 3. The morphological and physiological changes induced under Cd stress are responsible for variations in the accumulation of polypeptides during seed development (Sarry *et al*., 2006).

The effects of different Cd concentration levels on the two major subfractions (7S β-conglycinin and 11S glycinin) are shown in Figs. 5 to 12. The band numbers 1, 2, and 3 represented the α′ (~75 kDa), α (~65 kDa), and β (~50 kDa) in the 7S β-conglycinin, while the 11S glycinin comprised two types of polypeptides, i.e., acidic and basic, whose molecular weights fluctuate around 38kDa and around 20kDa, respectively (marked as band number 5, 6, and 7).

Fig. 5 displays the intensity difference in the polypeptides of the two primary protein subfractions in response to 10mg/kg concentrations (2 C-1) of Cd and various concentrations of ascobin. Here, the study found that ascobin has a little improvement effect on β-conglycinin α', α and β intensity. The α' and α band intensity in control plants was found to be 2.14 and 2.74, respectively. Compared to ascobin treated plants, α' and α band intensity were found 2.15, 3.27 (3 T-1), 2.70, 3.43 (4 T-2), 2.57, 3.45 (5 T-3), respectively. Similarly, the β intensity in control was recorded as 3.49 when compared to 4.04, 4.54 and 4.90 under 150mg, 300mg and 500mg ascobin treatment. However, more improvement was seen in glycinin acidic and basic subunit as shown in Fig. 6. In our set 1 C-0 the band intensity value of β-conglycinin α', α and β intensity noted 2.66, 3.0 and 3.66, which are high than first control (2 C -1) Similar results was seen in both glycinin subunits shown in Fig. 7 and all the raw value of band intensities of set 1 C-0 represent in Fig. 8. All these results explain even very low concentration of Cd i.e., 10mg/kg affects the soybean seed protein fractions and affect the band intensities on SDS-gel.

The polypeptide patterns of the two major seed protein sub-fractions in response to the 20mg/kg dose of Cd (6 C-2) and different levels of ascobin are shown in Fig. 9. In this set, the effect of ascobin was noteworthy; the major change was observed again in α' and α subunit of 7S β-conglycinin as compared to it β-subunit. In control conditions, the band intensities raw values of α' and α were 1.14 and 2.93, respectively, whereas, under three different ascobin treatments, the α' values were 1.84, 1.92, and 3.0 and α values were 3.65, 4.3, and 4.7. The changes in intensity peak value of β-subunit according to three levels of ascobin (150mg, 300mg and 500mg) was noted 4.15, 4.53, 4.76, respectively whereas in control it was 3.05, as shown in Fig.10. Here, we have found that at 20 mg/kg of Cd, ascobin improved the protein sub-fractions as compared to control. Fig. 3 and 4 on SDS-gel also supported that there was an apparent loss in intensity of band number 6 C-2, as compared to (7 T-1), (8 T-2), and (9 T-3).

The relative responses of the soybean seed protein subfractions to 30 mg/kg of Cd supply (10 C-3) are depicted in Fig. 11. Here, a loss in the peak intensity of 7S protein can be clearly seen, but very less impact observed on 11S glycinin. In this set, the impact of ascobin was also considerable but less than in the previous set, with 7S β-conglycinin exhibiting the greatest variation. In this group, the band intensities of the 7S, α', α and β at control (10 C-3) were 1.46, 2.32, and 2.4, respectively. Under the ascobin treatments, these band intensities changed to 1.57, 3.0, 3.8 (11 T-1), 1.55, 3.7, 4.4 (12 T-2) and 2.0, 3.34, 4.52 (13 T-3) as shown in Fig. 12. However, in 11S glycinin acidic and basic subunit little changes were observed in band intensity values under Cd and ascobin treatments. So, the effect of Cd under control-3 is much greater than under control-2 and control-1 on 7S β-conglycinin. The mitigating effect of ascobin on this third set was not that impres-

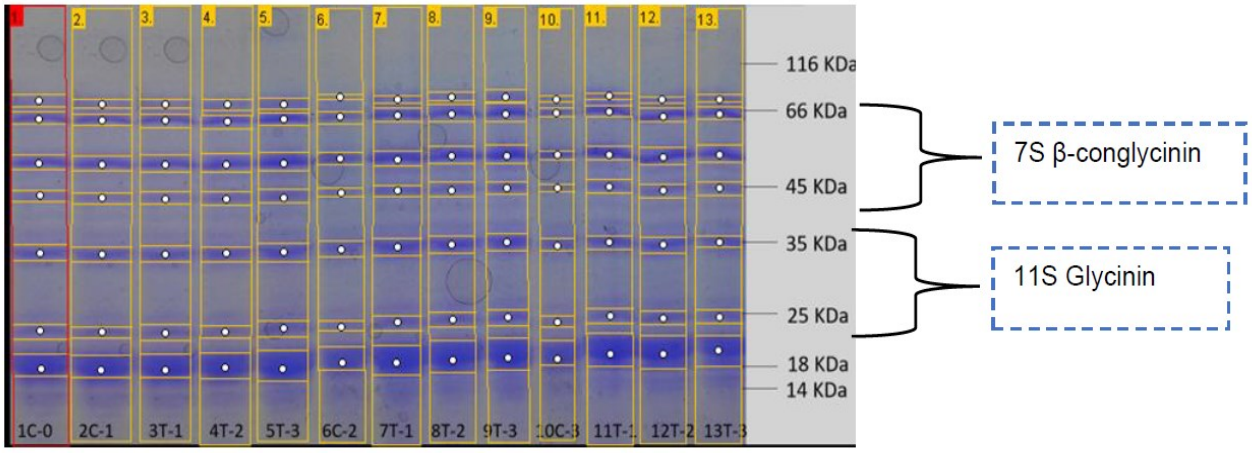

Fig. 4. *Showing soybean seed protein SDS-gel analysed by GelAnalyser for raw value of band intensities*

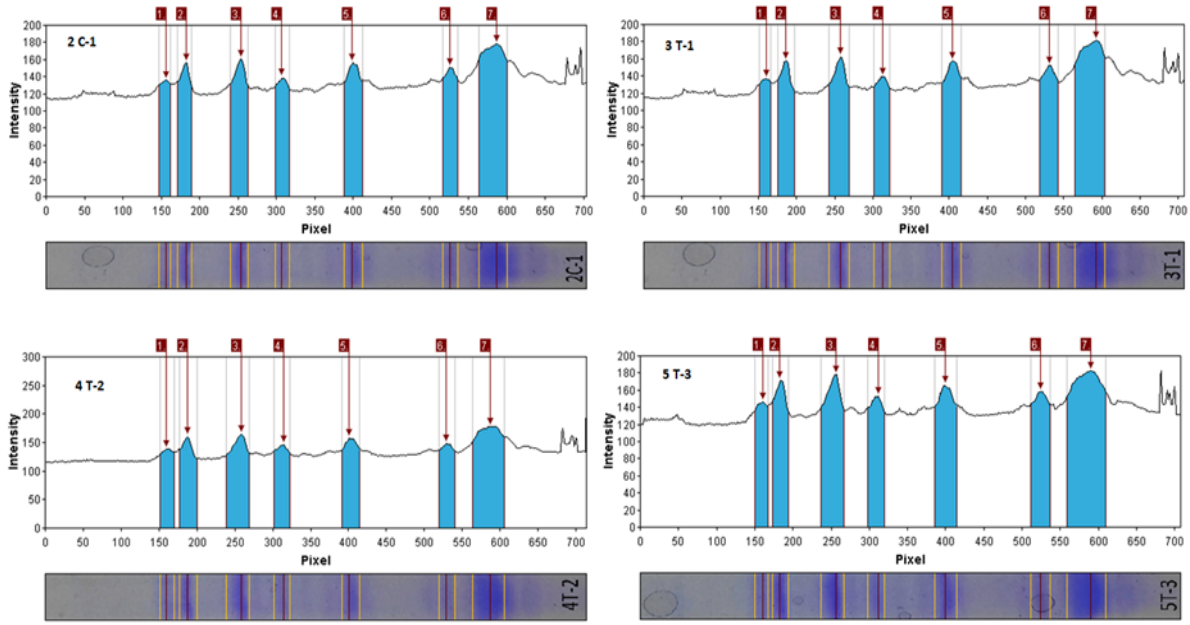

Fig. 5. *Lanes 2 C-1, 3 T-1, 4 T-2 and 5 T-3 showing the intensity peaks of different bands on SDS-gel of experiment set first*

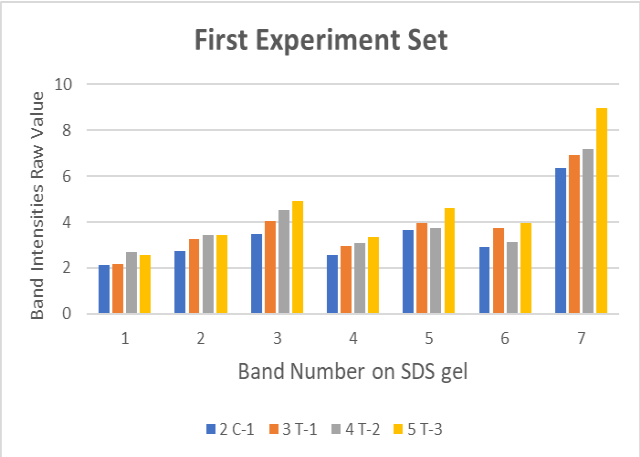

Fig. 6. *Lanes 2 C-1, 3 T-1, 4 T-2 and 5 T-3 showing the band intensities raw values of different bands on SDS-gel of 1st experiment set*

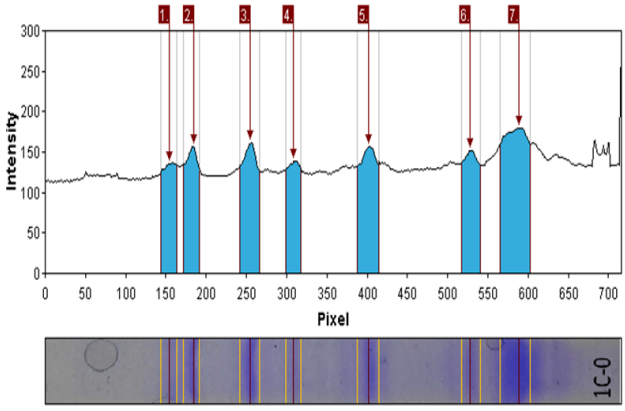

Fig. 7. *Lanes 1 C-0 showing the intensity peaks of different bands on SDS-gel*

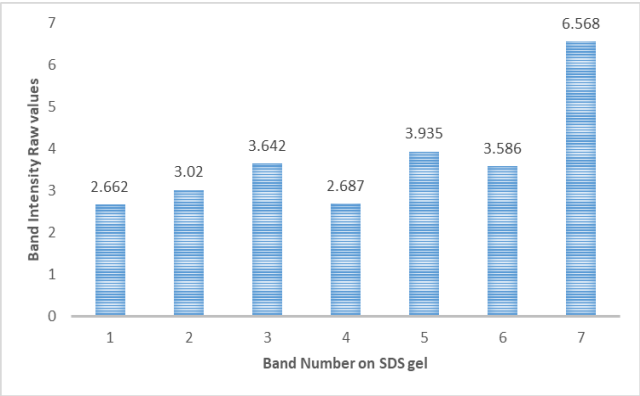

Fig. 8. *Raw value of 1 C-0 set band intensities of different bands on SDS-gel of Experiment set 1*

sive as compared to the previous two sets as shown in Fig. 12.

Due to the absence of sulfur-containing amino acids (methionine and cysteine), the 7S β-conglycinin lack disulfide linkages and has poor quality. Like other globulins, 7S globulin subunits are held together by hydrophobic and hydrogen-bonded interactions, as described by Thanh and Shibasaki (1977). Yaklich (2001) explained how β-conglycinin and glycinin, contribute to the enhanced seed protein content in soybean. Densitometric scanning of SDS-PAGE gel was used to estimate the subunits of these two major storage proteins. Soybean and other legume seed proteins are poor in sulphur-containing amino acids, and β-conglycinin has less sulphur-containing amino acid than glycinin (Yaklich, 2001). On SDS-PAGE, Ahsan *et al.* (2007) observed cadmium-induced alterations in the protein patterns of developing rice seeds. They discovered differences in protein patterns ranging from 116 to 45 kDa and 25 to 14 kDa, indicating that these are the primary and metal binding proteins in rice seed. In addition, they found that the polypeptide patterns of rice seeds showed significant alterations when exposed to elevated amounts of cadmium heavy metal. The present results also correlated with these finding, it was observed the higher Cd concentrations has a substantially greater negative effect on 7S β-conglycinin as compared to the 11S glycinin. This reduction in 7S β-conglycinin polypeptides can be seen in terms of their band intensity peak. Also, there was a clear enhancement in these subfractions with ascobin treatment and this is visible from band intensity peak yielded by gel-analyzer, i.e., lesser band intensity in control than the ascobin treated samples.

Conclusion

The present study on the impact of cadmium on protein sub-fractions in soybean's total seed proteins showed that cadmium decreased the seed protein quality by reducing the levels of the major globulin seed protein sub-fractions, i.e., 7S β-conglycinin and 11S glycinin. The loss of protein quality was restored to some extent with added ascobin. The polypeptide patterns exhibited alterations which were both qualitative and quantitative in nature. The observed changes were due to ascobin and Cd, which have induced the expression of some

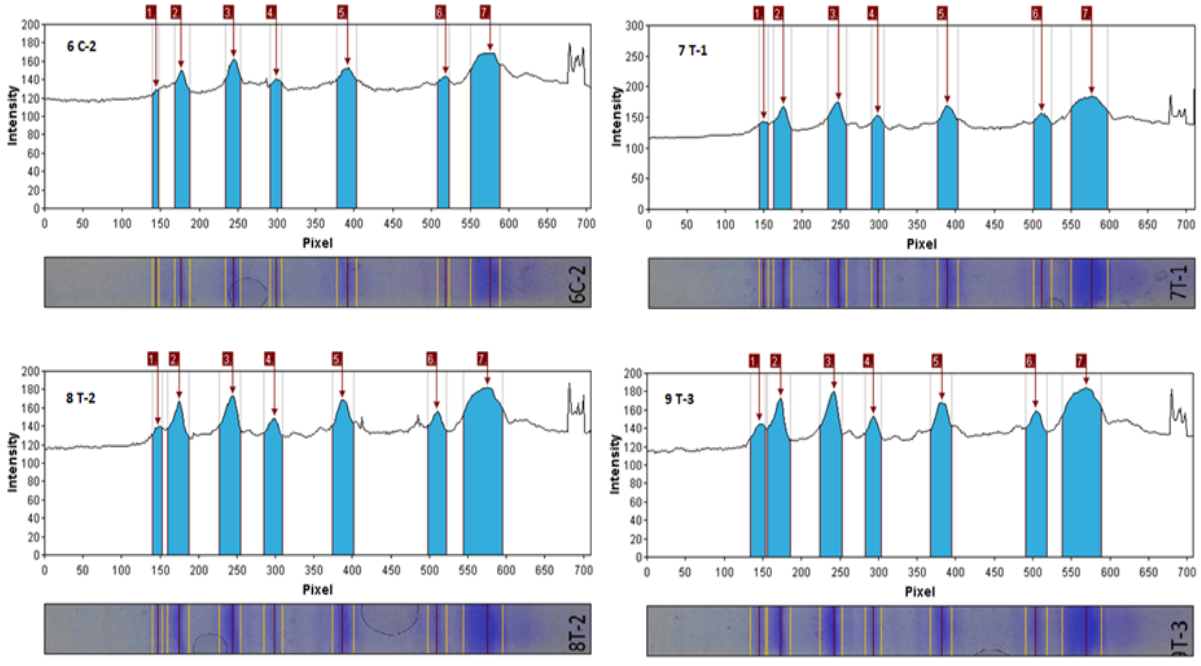

Fig. 9. *Lanes 6 C-2, 7 T-1, 8 T-2 and 9 T-3 showing the intensity peaks of different bands on SDS-gel of second experiment set*

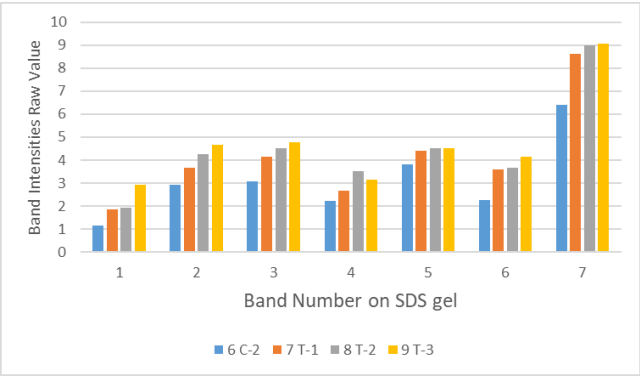

Fig. 10. *Lanes 6C-2, 7 T-1, 8 T-2 and 9 T-3 showing the band intensities raw value of different bands on SDS-gel of second experiment set*

previously dormant genes and the over-expression and down-regulation of genes encoding various polypeptides. Thus, it will be of significant importance and interest to extend investigations to molecular processes that govern such changes in soybean seed proteins. More research is required to develop soybean genotypes that can thrive under the Cd stress and produce enhanced yield and better quality and quantity of seed proteins.

ACKNOWLEDGEMENTS

The authors are thankful to the National Seeds Corporation Ltd. (NSC) for providing soybean seeds for our research work. NSC is a Schedule 'B'-Mini-ratna Cate-

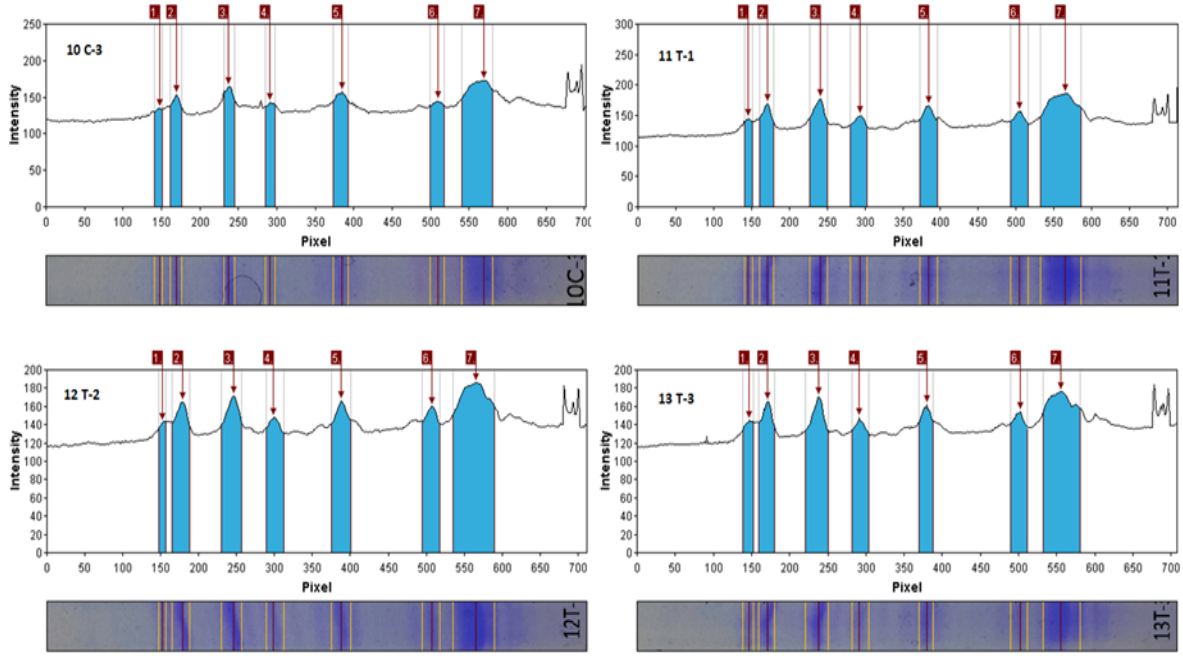

Fig. 11. *Lanes 10 C-3, 11 T-1, 12 T-2 and 13 T-3 showing the intensity peaks of different bands on SDS-gel of 3rd experiment set*

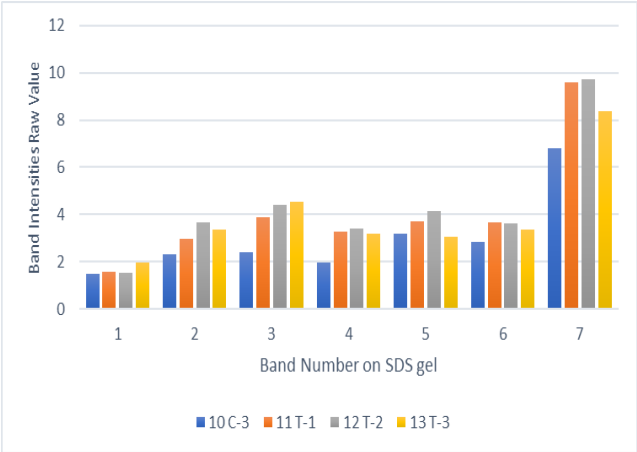

Fig. 12. *Lanes 10 C-3, 11 T-1, 12 T-2 and 13 T-3 showing the raw value of band peaks intensities of different bands on SDS-gel of 3rd experiment set*

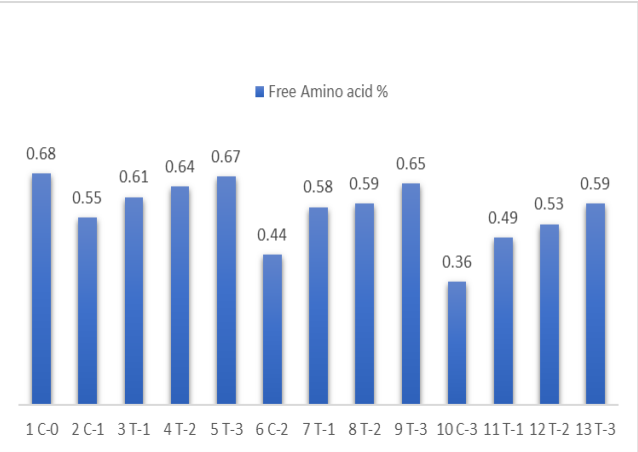

Fig. 13. *Percentage of free amino acids under different levels of cadmium and ascobin*

gory-I corporation completely owned by the Indian government and managed by the Department of Agriculture Cooperation and Farmer's Welfare, Ministry of Agriculture and Farmers Welfare. One of the authors (Amit) is also thankful to the University Grants Commission (UGC), New Delhi, for providing JRF fellowship for the successful completion of the present work.

Confilict of Interest

The authors declare that they have no conflict of interest.

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