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INFLUENCE OF SULFUR AND NITROGEN AVAILABILITY ON STORAGE PROTEIN ACCUMULATION AT DIFFERENT GROWTH STAGES IN MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK]

Arvind Kumar[1]*, Sruchi Devi[1], Sucheta Sharma[2], JS Sital[2], Sarvjeet Singh[3], Minhaj Ahmad Khan[1] and Surender Jangra[1]

[1]Department of Biochemistry, School of Bioengineering and Biosciences,
Lovely Professional University, Phagwara, Punjab-144411, India.
[2]Department of Biochemistry, [3]Department of Plant Breeding and Genetics,
Punjab Agricultural University Ludhiana, Punjab-141004, India.
* Author for correspondence: arvind_ldl@rediffmail.com

Abstract

In the present investigation, accumulation of various protein fractions at different stages of development in mungbean seeds of varieties PAU 911 and ML 818 harvested from plants grown under different treatments of sulfur (gypsum, single super phosphate) and nitrogen (urea, ammonium nitrate) either alone or in combination (urea + single super phosphate and gypsum + ammonium nitrate) was studied. The contents of protein fractions viz. albumin, globulin, glutelin, prolamin and subfractions of globulin (legumin and vicilin) increased under the effect of various treatments from 4 to 24 days after flowering (DAF) with maximum accumulation at 20 DAF as compared to control (T_1, without any sulfur and nitrogen source). Increased incorporation of radiolabelled leucine into albumin and globulin fractions of storage proteins in both the varieties was observed under the effect of different sources of sulfur and nitrogen. Maximum incorporation of C^{14} labelled leucine in storage protein was observed at 16 DAF (i.e. active stage of protein deposition in mungbean) and treatment with gypsum showed the highest incorporation of C^{14} labelled leucine in these protein fractions.

Key Words: Mungbean, storage protein fractions, sulfur and nitrogen nutrition, protein quality

Introduction

Mungbean (*Vigna radiata* L.Wilczek),also known as the green gram is the third most consumed pulse crop in India (Malik *et al.*, 2013; Chakraborty *et al.*, 2015; Mishra *et al.*, 2018). It occupies an area of 3.77 million hectare in India with 1.80 million tonnes production in the year 2010-2011 (Malik *et al.*, 2016; Mishra 2019a, 2019b). In Punjab, this crop was grown over 7.8 thousand hectares area with a production of 6.3 thousand tonnes with an average yield of 808 kg/hectare during 2010-2011 (Anonymous, 2012; Meenu *et al.*, 2016; Khan *et al.*, 2017).

Although pulses are rich source of proteins, yet they are generally deficient in sulfur containing amino acids, methionine and cysteine but rich in lysine and tryptophan, the limiting amino acids in cereals. Mungbean seed contains 22-25% protein, 1-2% fat, 55-60% carbohydrates and minerals, calcium and phosphorus being 118 mg and 340 mg per 100g of seeds respectively (Kaur *et al.*, 2016a, 2016b; Kaur *et al.*, 2020). It is considered as a substitute of animal protein and forms a balanced diet when used along with cereals (Consideine, 1992; Singh *et al.*, 2018; Barman *et al.*, 2019). Mungbean has a special importance in intrinsic crop production system of the country for its short growing period (Ahmed *et al.*, 1978), thus can be cultivated twice a year. Mungbean cultivation is popular among resource-poor farmers because it does not require lot of water or other inputs and helps to restore soil fertility through symbiotic nitrogen fixation (Prabhakar *et al.*, 2013; Chauhan *et al.*, 2017; Gaikwad *et al.*, 2018).

In higher plants, large amounts of storage proteins accumulate during period of seed development and maturation, which are subsequently used as carbon and nitrogen sources for energy purposes during seed germination (Bewley and Black, 1994; Singh *et al.*, 2019; Prabhakar *et al.*, 2020). Globulin, the salt soluble protein fraction constitute about 70-80% of total seed protein in mungbean and other seed storage protein fractions include albumin, glutelin and prolamins. Globulins are further classified into two type's viz. legumin like (11-12S) and vicilin like (7-8S) (Derbyshire *et al.*, 1976) and are present in the ratio of 65:35 in mature seeds (Sital and Narang, 1994; Kumar *et al.*, 2019). The accumulation of globulins was active during 9-18 days after flowering (DAF), with maximum accumulation rate near 15 DAF. Albumins from pulses are generally high in sulfur containing amino acids whereas legumin proteins generally have high amounts of methionine and cysteine compared to vicilin fraction (Mendoza *et al.*, 2001). A seed storage protein is an important agronomic trait, playing a crucial role in supplying the essential amino acids to human and domesticated animals (Poxleitner *et al.*, 2006).

Seed protein composition and quality can be altered by environmental factors such as temperature, nutrient etc. Grain legumes can modulate their seed storage protein composition in response to sulfur and nitrogen (Tabe *et al.*, 2002). Plants grown in abundant sulfur can accumulate sulfur rich proteins to high levels with improvement in the protein amino acid balance in legume seeds (Gusain *et al.*, 2015). Studies on protein quality and quantity improvement under the influence of various minerals have been reported in soybean (Sexton *et al.*, 1998), cowpea (Evans *et al.*, 2006), lentil (Al Karki 1999, Sital *et al.* 2011) and chickpea (Ghalotra *et al.,* 2007). Both sulfur and nitrogen are significant supplements required by crops for proteins structure, nutrients, development controllers and other auxiliary segments (Leustek and Saito 1999, Marshner, 1995) and improve plant development and yield moreover. In the present examination endeavors have been made to watch the impacts of sulfur and nitrogen sustenance alone or their synergistic consequences for protein quality in growth of mungbean (*Vigna radiata* L. Wilczek) seeds.

Materials and Methods

Mungbean crop assortments PAU 911 and ML 818 suggested for development were planted in the test fields of pulses area, department of Plant Breeding and Genetics, PAU Ludhiana in Random Block Design with three replications. Before sowing, soil status for nitrogen and sulfur was determined. Soil was then enhanced with various sulfur and nitrogen sources @40kg/ha as T1 (control where neither sulfur nor nitrogen was included), T2 (single super phosphate), T3 (urea), T4 (single super phosphate and urea), T5 (gypsum), T6 (ammonium nitrate) and T7 (gypsum and ammonium nitrate) (Vyas, 2017; 2019; Sudhakar *et al.*, 2015).

After sowing, uniform plants were randomly selected and flowers were tagged. Pods were collected at an interval of 4 days after flowering (DAF) and brought to laboratory under refrigeration conditions. Seeds of uniform size at each developmental stage were screened, air dried, powdered and stored in air tight glass containers for subsequent biochemical study. Distinctive protein parts were removed from mungbean seed flour dependent on their solvency at 25°C in refined water (for egg whites), 0.2 M sodium phosphate cushion pH 8.0 containing 3% NaCl (for globulin), 0.1 M NaOH (for glutelin division) and 70% ethanol (for prolamin) following the method given by Pant and Tulsiani (Pant and Tulsiani, 1969). The globulins were fractionated into legumins and vicilins by repeated isoelectric precipitation from crude globulins at pH 4.6 (isoelectric point legumins) (Basha and Beevers, 1975). The content of protein of different fractions viz. total salt extractable proteins, albumins, globulins, vicilins and legumins were determined by the method of Lowry *et al.* (Lowry *et al.*, 1951).

The radiolabeling studies were conducted by the modified method of Spencer (Spencer *et al.*, 1980). Seed cotyledons were placed flat on the drops containing 20 µl (3.57 µCi/20 µl) of the working solution (140 µl of stock diluted with equal volumes of double distilled water in disposable petriplate. Incubation for 12 h and 24 h were given to the samples kept in triplicate under the fluorescent lamp at a distance of 40 cm from samples plates. After incubation the samples were rinsed well with distilled water. From radiolabelled cotyledons globulin and albumin protein fractions were extracted by the procedure mentioned above (Lowry *et al.*, 1951). To 100 µl of each sample 5 ml of liquid scintillation solution (cocktail) was added and radioactivity was measured using liquid scintillation counter (Perkin Elmer). There were three replications with duplicate observations for each replication and data were analyzed for the critical difference between treatments and days after flowering (DAF) at 5% level of significance.

Results and Discussion

Application of various sources of sulfur (T_2 & T_5) and nitrogen (T_3 & T_6) alone or in blend fundamentally expanded the albumin content in seeds of PAU 911 and ML 818 in all the treatments (except T_2) as compared to T_1 (control) at different stages of development (Fig. 1). The albumin content did not differ significantly up to 8 DAF between treatments T_2 and T_6 in seeds. Albumin content in developing seeds of both varieties increased significantly from 4 DAF to 20 DAF in all the treatments. The globulin content increased from 0.14 mg (4DAF) to 5.50 and 4.92 mg (24 DAF) in control seeds of PAU 911 and ML 818 seeds respectively (Fig. 1). The globulin content increased significantly at different stages of development in PAU 911 and ML 818 seeds under the influence of either source of sulfur and nitrogen or when supplied in combination. Maximum increase in albumin and globulin contents was observed in developing seeds of both the varieties at 20 DAF under T_5 treatment.

Sulfur and nitrogen supply alone (T_2, T_3, T_5, T_6) or in combinations (T_4, T_7) significantly increased the glutelin and prolamin content from 4 DAF to 20 DAF in both the varieties as compared to control (T_1) with maximum increase in their contents under T_5 treatment (Fig. 2).

The significant proteins in seeds of different vegetables were represented by globulins, trailed by albumins, glutelin and prolamins being the most reduced as announced before (Dhanker *et al.*, 1990). Globulins speak to 70-80% of all out seed stockpiling protein and are combined during seed improvement on polysomes, moved through lumen, sequestered lastly saved in particular compartments termed protein storage vacuoles, which is defined by the presence of α- and β- tonoplast intrinsic protein in their tonoplasts (Jiang *et al.*, 2000; Kar *et al.*, 2018). Both globulins and albumins contents increased in chickpea with increasing concentrations of nitrogen, sulfur and potassium (Kumar and Matta 1997, Singh and Matta 2005). Different sulfur sources viz. single super phosphate, gypsum and elemental sulfur increased the albumin and globulin content in chickpea seeds at all stages of development with maximum effect shown by gypsum followed by single super phosphate and elemental sulfur (Ghalotra *et al.*, 2007). Gillepspie *et al.* reported that seed globulin composition (α and γ conglutins) in lupin seeds was greatly decreased during S-deficiency and level of congluten β, which normally contain no methionine and low cysteine was increased to maintain the protein level. Our results are in agreement with the previous studies done on other pulses (Sital *et al.* 2013, Ghalotra *et al.*, 2007, Singh and Matta 2005) that supplement of different minerals can increase the quality of storage protein fractions in these crops.

The legumin and vicilin content increased significantly under the effect of various treatments of sulfur and nitrogen either alone or in blends in developing seeds of mungbean (Fig. 3). The maximum value of both legumin and vicilin content was found under the effect of T_5 (gypsum) at 20 DAF among all the treatments. The changes in legumin and vicilin content followed the similar trend as observed with globulin fraction. Legumin and vicilin ratio was about 35:65 at different developmental stages in PAU 911 and ML 818. Earlier studies also reported 1:2 ratios for legumin and vicilin in legumes (Sital and Narang, 1994; Nankar *et al.*, 2017).

The increase in storage protein contents were less when SSP, urea and ammonium nitrate were supplied alone in comparison to when given in combination (Figs. 3-5). However, gypsum as a source of sulfur showed maximum increase in their contents. Storage protein content of both the varieties was almost similar when nitrogen and sulfur sources were applied in combinations. Sulfur and nitrogen nutrition regulate the sulfur assimilation pathway in plants (Chiaiese *et al.*, 1989). In comparison to nitrogen, sulfur deficiency derepresses the expression of sulfur transporters in roots responsible for its uptake (Takahashi *et al.* 2000) and increases the expression of enzymes of sulfur reduction and assimilation pathways (Saito, 2000). Addition of nitrogen upgrades root advancement which improves the inventory of different supplements and water to the developing pieces of the plants, bringing about an expanded photosynthetic zone and in this way dry matter amassing (Ali *et al.*, 2010; Prasher *et al.*, 2018; Sharma *et al.*, 2019).

Radiolabelled studies showed maximum incorporation of C^{14} labeled leucine at 16 DAF in albumin as well as in globulin fraction in seeds from both varieties after incubation for 12/24 h period (Table 1). Gypsum was found to be most effective among all the treatments of sulfur and nitrogen and showed maximum deposition of C^{14} labeled leucine in albumin and globulin fraction of cotyledons from PAU 911 and ML 818 seeds at 12 h incubation period. Similar observations were made after 24 h incubation period (data not given). The level of incorporation of radiolabelled leucine was higher in globulin fraction as compared to albumin fraction of protein in both the varieties. In earlier studies on other crops it was also observed that 80% of the ^{3}H-leucine incorporated into globulin protein fraction in broad bean (Bailey *et al.*, 1970). Incorporation of labeled amino acids in storage proteins in developing mungbean (*V. radiata* (L.)) seeds showed that storage protein synthesis was maximum at 10 DAF (Dhillon and Nainawatee, 1990). The albumins were synthesized first followed by globulin and glutelin fractions. Zheng *et al.* found that storage protein content increased from 25 to 55 DAF and then declined towards later stages. The deposition of radiolabelled leucine in total protein, albumin and globulin fractions of chickpea seeds was maximum when gypsum was sole source of supplied sulfur in chickpea var. PBG1 and var. BG1053 and maximum storage protein deposition was reported from 27 DAF to 32 DAF (Ghalotra, 2007).

During seed development, the synthesis and accumulation of various seed storage protein fractions can be affected by number of environmental factors. As most of the storage protein fractions are deficient in sulfur containing amino acids, the results of present studies suggest that protein quality can be improved by supplying gypsum to check their deficiency in legumes.

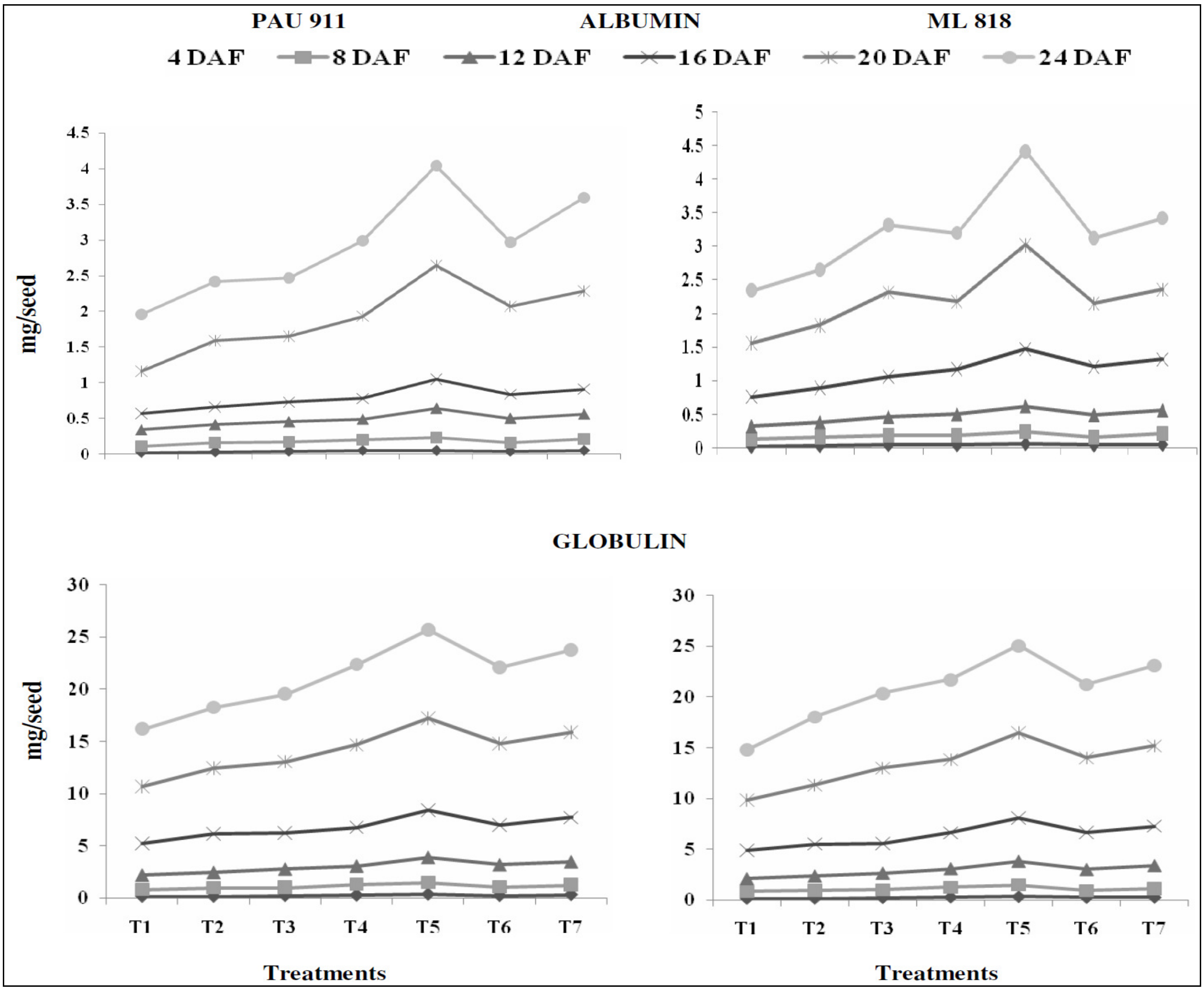

Fig. 1: Effects of various treatments of sulfur and nitrogen on albumin and globulin content of mungbean seeds of variety PAU 911 and ML 818 at different stages of development.
Treatments @40kg/ha: T_1 - Control; T_2 - Single Super Phosphate (SSP); T_3 - Urea; T_4 - Single Super Phosphate (SSP) + Urea; T_5 - Gypsum; T_6 - Ammonium Nitrate ; T_7 - Gypsum + Ammonium Nitrate

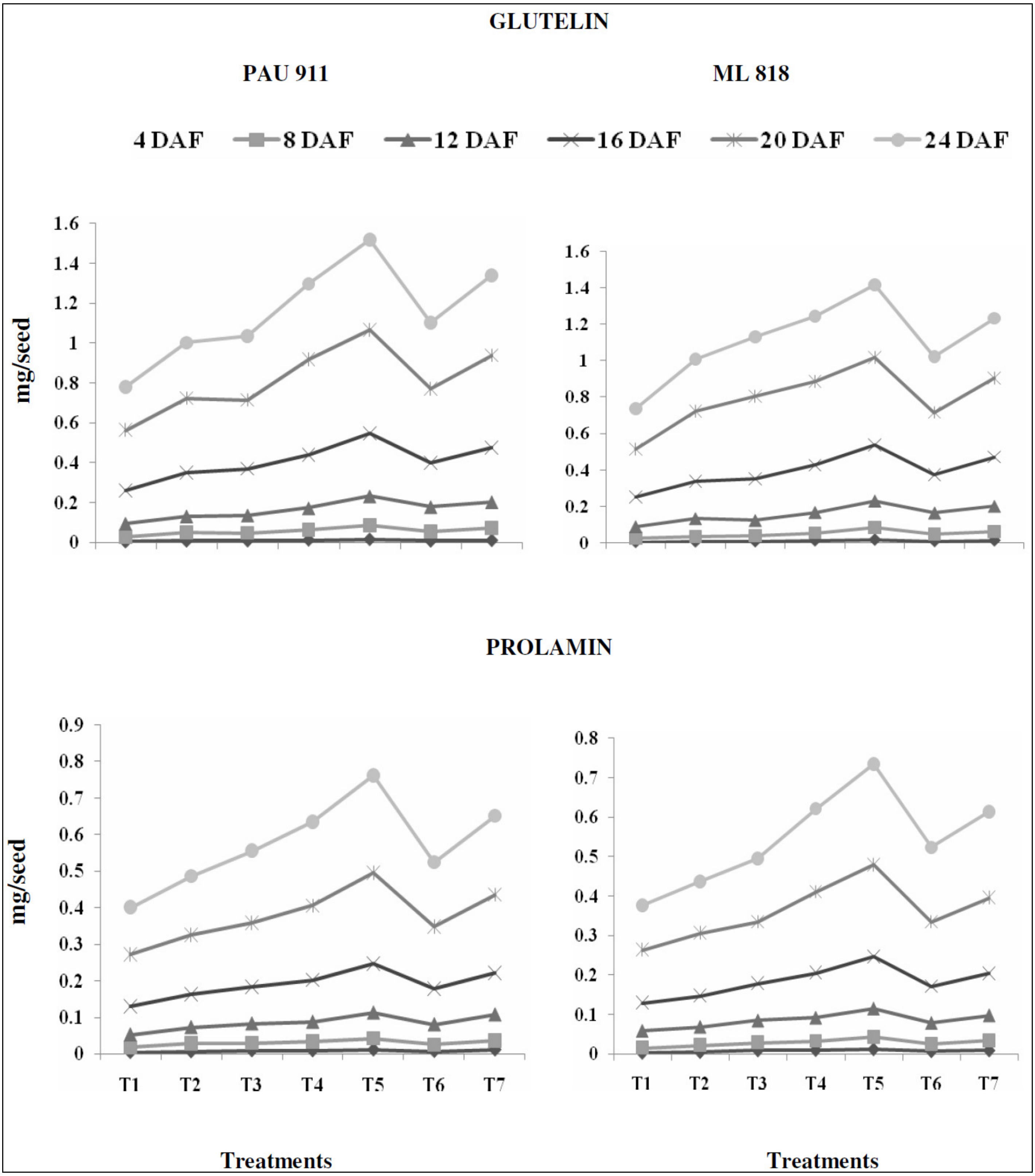

Fig 2: Effects of various treatments of sulfur and nitrogen on glutelin and prolamin contents of mungbean seeds of variety PAU 911 and ML 818 at different stages of development.

Treatments @40kg/ha: T_1 - Control; T_2 - Single Super Phosphate (SSP); T_3 - Urea; T_4 - Single Super Phosphate (SSP) + Urea; T_5 - Gypsum; T_6 - Ammonium Nitrate ; T_7 - Gypsum + Ammonium Nitrate

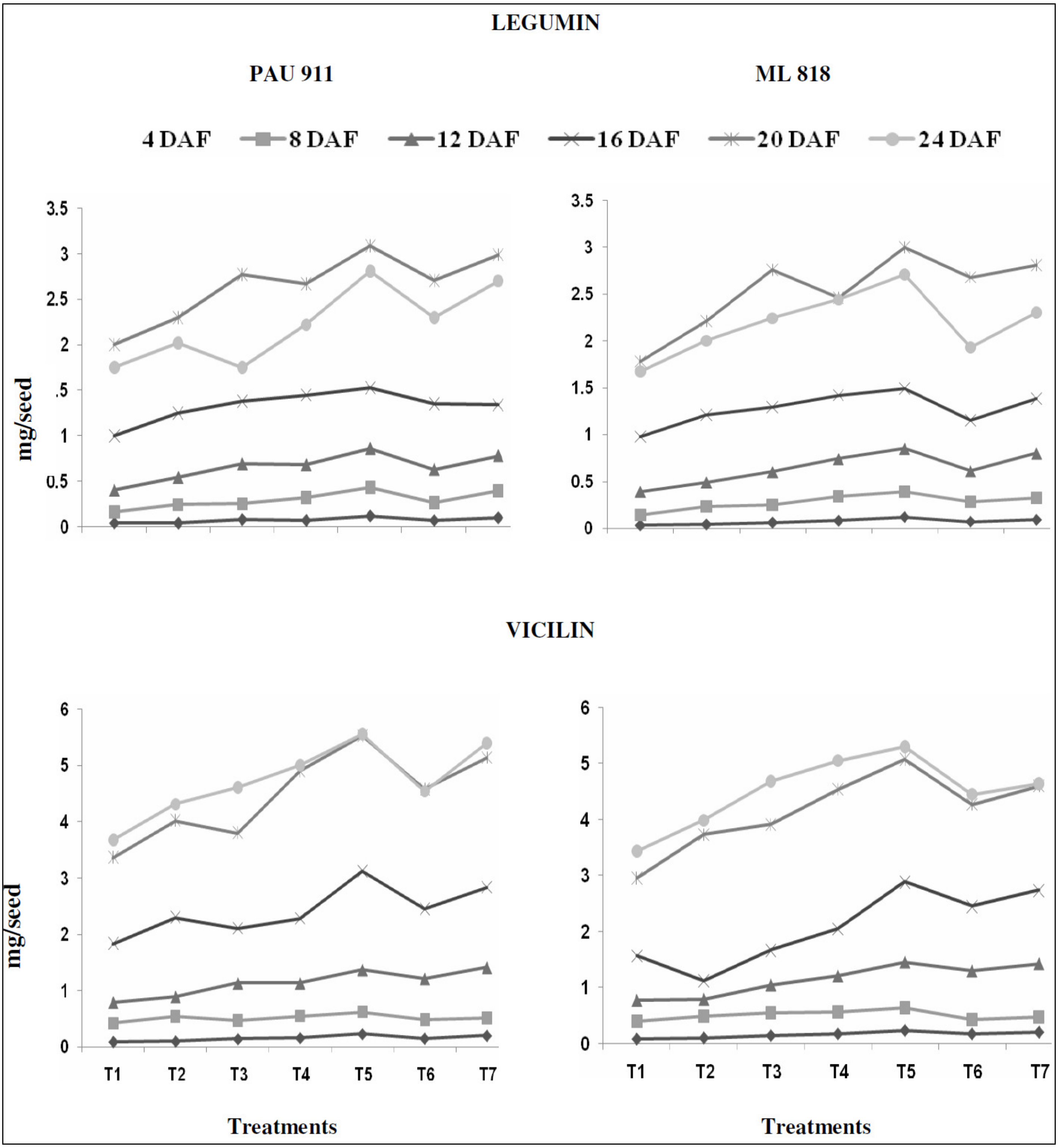

Fig 3: Effects of various treatments of sulfur and nitrogen on legumin and vicilin content of mungbean seeds of variety PAU 911 and ML 818 at different stages of development.

Treatments @40kg/ha: T_1 - Control; T_2 - Single Super Phosphate (SSP); T_3 - Urea; T_4 - Single Super Phosphate (SSP) + Urea; T_5 - Gypsum; T_6 - Ammonium Nitrate ; T_7 - Gypsum + Ammonium Nitrate

Table 1: Effect of various treatments of sulfur and nitrogen on incorporation of C^{14} labelled leucine in albumin and globulin protein fractions (CPM/g fresh weight) of mungbean seeds of variety PAU 911 and ML 818 after 12 h incubation period.

Treatments	PAU 911			ML 818		
	12 DAF	16 DAF	20 DAF	12 DAF	16 DAF	20 DAF
	Albumin					
T_1	614.39	802.46	796.31	602.39	791.16	702.34
T_2	728.62	907.51	872.42	698.34	840.46	786.29
T_3	746.12	930.45	922.14	724.13	856.39	809.34
T_4	954.14	1140.91	1102.56	901.92	1076.46	948.56
T_5	802.34	840.56	832.42	802.16	946.31	876.42
	Globulin					
T_1	748.12	946.12	904.34	726.29	902.19	846.49
T_2	946.92	1146.34	1072.42	902.76	1096.76	972.34
T_3	992.18	1259.96	1197.00	912.81	1143.21	1102.34
T_4	1236.12	1415.02	1339.49	1136.46	1316.19	1301.46
T_5	1032.92	1211.42	1142.38	982.32	1134.39	1102.16

Critical Difference (P<0.05)	Albumin		Globulin	
	PAU 911	ML 818	PAU 911	ML 818
Teatments-A	2.91	3.86	5.77	5.78
DAF-B	2.26	2.97	4.47	4.47
A x B	5.05	6.65	9.99	10.00

All values are means of triplicate observations.

Treatments: @40kg/ha: T_1 - Control; T_2 - Single Super Phosphate (SSP); T_3 - Urea; T_4 - Gypsum; T_5 - Ammonium Nitrate

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