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Effect of asparagine or glutamine on growth and metabolic changes in *phaseolus vulgaris* under *in vitro* conditions

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Effects of L-asparagine and L-glutamine on growth, pigments content and metabolism of *Phaseolus vulgaris* plants, at two stages of plant growth and development in was investigated in micropropagated tissues (*in vitro*). In general, all growth parameters increased by 1 & 2 mM asparagine or glutamine treatments while decreased in response to other concentrations (3, 4 & 5 mM), Similarly total carbohydrates and its fractions also increased in response to low concentrations whereas the higher concentrations decreased these parameters, throughout the experiment. The pattern of changes in chlorophyll a, chlorophyll b and carotenoids were more or less similar to that in carbohydrates in response to asparagine or glutamine treatments. As compared with untreated plants, treatment with all concentrations of asparagine or glutamine induced marked increases in amide nitrogen, total nitrogen and protein whereas ammonia nitrogen, peptide nitrogen and total soluble nitrogen, decreased during seedling and vegetative stages. Ions content (K^+, Na^+, Ca^{++} and Mg^{++}) increased significantly by 1 mM asparagine or glutamine and markedly decreased by the other concentrations. Asparagine and glutamine treatments increased and decreased the growth promoters (auxins, gibberellins and cytokinins) level at low and high concentrations, respectively. On the other hand a reverse situation was observed in case of ABA content. In general, the activity of all the determined enzymes (asparagine synthetase, glutamine synthetase, nitrate reductase and protease) was decreased by increasing asparagine or glutamine concentrations, during the two stages of *Phaseolus vulgaris* growth and development.

Key words: Amide, French bean, Ions, Hormones, Enzymes

Cell and tissue culture techniques of plants provide alternative research material, especially for developmental and metabolic studies that might be difficult to conduct in intact plants (Dudits *et al*. 1995). The ability to investigate the physiological effects of nutrients, plant hormones and other chemical constituents in defined culture media under controlled conditions is one of the most important applications of tissue culture (Kärkönen, 2001).

All living organisms require nitrogen as an indispensable element, most of which is bound up in amino acids and nucleotides (Sungdae, 2002 and Tabatabaei *et a*l. 2008). Most living organisms are capable of assimilating ammonia into the amino-group of glutamine, which is the direct source of nitrogen for the building blocks of macromolecules and other biological compounds (Sungdae, 2002; Lehmann and Ratajczak, 2008). It has been reported that addition of glutamine to medium containing cytokinins or cytokinins and auxins increased the regenerative ability of old calluses of wheat by 6 to 10% and a shoot induction frequency by 55% (Shrivastava and Chawla 2001). Moreover, for mature zygotic embryos, the addition of asparagine, glutamine or arginine to the basal LPM culture medium has resulted in improved somatic embryogenesis induction (Vesco *et al*. 2001).) Ammonia and glutamine can serve as alternative nitrogen sources although, at high concentrations (≥ 1mM), they can inhibit growth (Zhang *et al*. (1999). Further, asparagine could act as an amide group donor similar in role to glutamine (Schubert, 1983). In germinating seeds of legumes, the amino acids are catabolized by

both glutamate dehydrogenase (GDH) and transaminases. Ammonium is reassimilated by glutamine synthetase (GS) and, through the action of asparagine synthetase (AS), is stored in asparagine (Asn) (Lehmann and Ratajczak ,2008)

Nitrogen enrichment has been reported to stimulate net dry matter production in *Dactylis glomerate* L plan (Harmens *et al.* (2000). Menéndez *et al*. (2002) observed that nitrogen application was followed by an increase in chlorophyll content after 4 days of treatment and this was followed by an increase in biomass after 10 days in *Chaetomorpha linum*.. On the other hand, Tremblay *et al*. (1999) reported that when *Phaseolus vulgaris* and sweet corn grown at different nitrogen concentrations, chlorophyll contents of both were not correlated to nitrogen application. Whereas, Martin *et al*. (2002) detected significant reduction of *Arabidopsis* seedling chlorophylls, by low nitrogen treatment (0.1 mM). Addition of nitrogen to the growth medium has been report to increase the cations in *Lolium multiflorum* (Sagi *et al*. 1998). Moreover, Kubik-Dobosz and Buczek (1999) stated that when *Pisum sativum* plants supplied with 1mM glutamine or asparagine took up ammonium and potassium at rate lower than those of control plants. The efflux of NH_4^+ and K^+ from root to ambient solution was enhanced under these treatments.

The relations between nitrogen metabolism and endogenous hormones are reciprocal. Not only do these hormones control certain phases of protein synthesis and degradation, but two of the three main classes of hormones; auxins and cytokinins, are themselves nitrogen containing compounds whose production is inevitably linked with the nitrogen metabolism of the plant (Luckwill, 1967). Moreover, glutamine synthetase is the key enzyme in ammonia assimilation and catalyzes the ATP-dependent condensation of NH3 with glutamate to produce glutamine (Ortega *et al.* 1999). In this connection, Miflin and Habash (2002) reported that, glutamine synthetase is necessary for the biosynthesis of nucleic acids, proteins, complex polysaccharides, and various coenzymes. Ogawa *et al*. (1999) proved that after addition of glutamine to nitrate-containing medium, nitrate reductase activity was repressed in cultured *Spinach* cv. Hoyo cells. On the other hand, Li *et al*. (2001) found that in maize seedlings, 5mM glutamine strongly reduced nitrate reductase activity in the roots, however, in shoots no significant effect was detected. Moreover Niharika *et al*. (1998) reported that the uptake of nitrate by intact seedlings was inhibited by glutamine, whereas the total protein content of excised organs was unaffected.

In this view, the objective of the present investigation was to find out the possible effects of the two different amide- nitrogen compounds (asparagine and glutamine) on growth, pigments,, carbohydrate, nitrogen, protein, ions and growth regulators contents in micropropagated tissues of French bean (*Phaseolus vulgaris*). In parallel, changes in the activity of some related enzymes i.e. asparagine synthetase (AS), glutamine synthetase (GS), nitrate reductase (NR) and protease were also documented.

MATERIALS AND METHODS

Cultural conditions

Preparation of explants: In tissue culture techniques, seeds were rinsed in 80% ethanol for 3 min. and then surface sterilized for 20 min using 20% sodium hypochlorite supplemented with a drop of Tween-80 according to the method of Rodriguez *et al*. (1990). The seeds were thoroughly rinsed with distilled water 3 times and germinated on agar; 0.8% (w/v), in glass jars at 28 ^{o}C±0.1 in the dark. For multiplication and proliferation; all steps were carried out under sterilized conditions. Explants were prepared by removing the seed coat and slicing the embryonic axis into two halves while still attached to the cotyledons The epicotyls and hypocotyls were removed 1 mm from the cotyledonary node in such a way that the explants contained one cotyledon and a small portion (2-3 mm) of the split embryonic axis attached to it (Chandra *et al.* 1991). Explants (5 per each jar), were cultured in glass jars containing 100ml of the culture medium.

Culture media: The preparation is according to Murashige and Skoog (1962) (MS) with addition of 20 µM benzyl aminopurine and 2 µM naphthalene acetic acid (Allavena and Rossetti, 1986) The pH of the medium was adjusted to 5.8 with NaOH or HCl before the dissolution of agar. The jars were housed in a culture room at light intensity of 40µ EM2 s-1 photon flux density supplied by six lamps Philips with 16 hours photoperiod at

25 ± 1.0 ^{o}C and maintained under such conditions for a period of 7 days. Explants that had been previously proliferated, was dissected and cultured on the media pretreated with filter sterilized asparagine or glutamine which were added at the desired concentrations (1, 2, 3, 4, 5 mM) and were allowed to grow under the same conditions as described before. At 6 and 15 days intervals which represent seedling and vegetative stage, respectively, micropropagated tissues were harvested for determination of shoot growth parameters (height, fresh weight, dry weight and water content), analysis of metabolites (pigments content, carbohydrate contents, ions content, nitrogen contents, protein content) as well as hormonal content. In addition, activities of asparagine synthetase, glutamine synthetase, nitrate reductase and protease enzymes were determined.

Estimation of photosynthetic pigments: The protocol for measurements of the plant photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) was based on methods of Arnon (1949) for chlorophylls and Horvath *et al*. (1972) for carotenoids as adopted by kissimon (1999).

Estimation of carbohydrates: Total soluble sugars and sucrose were extracted and determined using modifications of the procedures of Yemm and Willis (1954) and Handel (1968), respectively. The method used for estimation of polysaccharides in the present study was that of Thayermanavan and Sadasivam (1984).

Estimation of nitrogenous constituents: The method used in this study was essentially that adopted by Yemm and Willis (1956). Ammonia-N was estimated spectrophotometrically by the method of Delory (1949) using Nessler's reagent as modified by Naguib (1964). The method used for estimation of amide-N was that recommended by Naguib (1964). Peptide nitrogen was estimated according to Kwon *et al.* (2000). The total soluble nitrogen and total nitrogen were determined by the conventional semi micro modification of Kjeldahl method (Pirie, 1955 and Chinbal *et al*. 1943 respectively).

Estimation of protein: The method of protein extraction was that of Scarponi and Perucci (1986).and determined spectrophotometrically by Bradford (1976) method.

Estimation of ions: Flame photometer was used for determining potassium and sodium, while calcium and magnesium were measured by atomic absorption spectrophotometer according to the method described by Chapman and Pratt (1978).

Determination of enzyme activities:

Determination of As activity: The method used in the present study was essentially that of Ravel (1970) where the enzyme is most easily measured by substituting hydroxylamine for ammonia then the amount of aspartyle hydroxamic acid formed is determined colorimetrically with ferric chloride reagent .

Determination of Gs activity: The method used in this investigation is as described by Sadasivam and Manicham (1992).Glutamine synthetase synthetase catalyses the γ-glutamyl transfer reaction. Hence, it can be assayed by measuring the production of γ-glutamyl hydroxamate . γ- glutamyl hydroxamates reacts with ferric chloride to produce a brown colour in acidic medium. When the activity is measured in the presence of Mn++, it represents total glutamine synthetase activity (adenylated and unadenylated form may be measured by inhibiting the adenylated form by the addition of 60mM Mg^{++}.

Determination of NR activity: The method of Hageman & Reed (1980) was applied.

Determination of protease activity: The method of Colowick *et al*. (1951) was used .The assay of protease activity was performed as described by Basha and Beevers, 1975 and Salmia *et al*. (1978).

Extraction, separation and bioassay of growth bioregulators: Extraction, separation were performed according to Shindy and Smith (1975). Auxins (IAA) were bioassayed by using the straight –growth test of *Hordeum vulgare* cv. 'Giza 118' coleoptile sections Foda and Radwan (1962). Gibberellins (Gas) were bioassayed by the growth of *Lactuca sativa* (cv.'Roumine') hypocotyls, which can be used to bioassay a number of Gas and GA- like subustances Frankland and Wareing, 1960 ; Crozier *et al.* 1970.

Cytokinins was bioassayed by assessing the growth of the cotyledon tissue of Xanthium brasilicum seeds, which expresses a rapid cytokinin response which can be obtained in solutions of very small volumes (Esashi and

Leopold (1969). ABA was bioassay by using *Triticum aestivum* L. grains, which were germinated in the dark for 70h at 25°C, according to the procedure used by Wright (1956).

The full data of the different treated micro propagated tissues were statistically analyzed and comparison among means (three samples) was carried out using Statgrapfic – vers-4-2-Display (one - tailed ANOVA) as described by Snedecor and Cochran (1980).

RESULTS & DISCUSSION

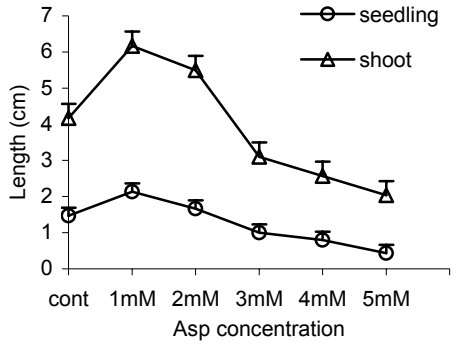
In recent years much attention has been given both to cost-effective micropropagation and to medicinal plants as sources of curare compounds for several ailments. Propagation through axillary bud multiplication is an easy and safe method for obtaining uniformity and it also assures the consistent production of true-to-type plants within a short span of time (Salvi *et al.* 2001).

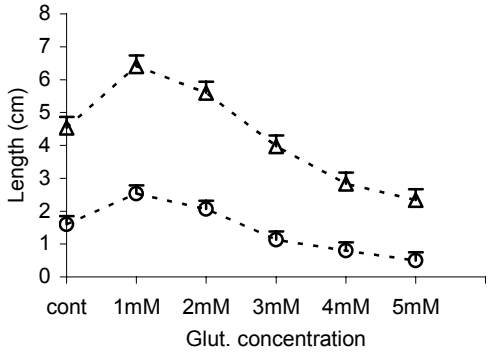
Changes in growth parameters: Perusal of the data in figures 1 a & b indicated that the different growth parameters (vegetative length, fresh& dry weights and water content), *in vitro*-cultured tissues of French bean showed significant increase by 1 mM asparagine or glutamine. In this connection, Green *et al*. (1990) found that amides were an acceptable nitrogen source for increased growth rate of shoots after 12 weeks in vitro of *Prosopis alba*. Thus, it can be stated that in the present study adequate amount of amides (1mM asparagine or glutamine) after being hydrolyzed in the medium, were taken up in the tissues as ammonia, and consequently assimilated within french bean micropropagated tissues and hence caused the observed increase in the various growth parameters. This increase may thus be directly related to the increased flux of amide to the leaf and/or to the subsequent reduction processes involved (Sutherland *et al.* 1985). In addition, the amides have been reported to induce effects like stimulation of cell wall formation, elongation of cells and increased cell division (Baker *et al*. 1997; Schröder *et al*. 2005).

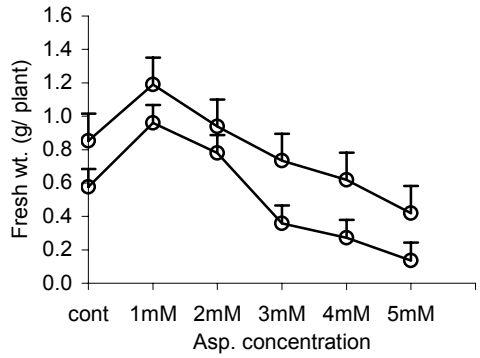
In present study, 2 mM glutamine or asparagine treatments did not significantly influenced the growth parameters. In support, Shetty *et al*. (1992) studied the stimulation of shoot organogenesis in glycine max by amide treatments and stated that, glutamine and asparagine resulted in poor multiple shoot formation. In present study, treatment with 3, 4 and 5 mM significantly decreased the above mentioned parameters. In accord with these results, Geisler (1985) has also reported a general reduction in leaf area, and root surface area with increasing nitrogen concentration in maize, spring barley and field beans. Also, Zhang *et al*. (1999) assured that the growth of Arabidopsis could be inhibited by using high concentrations of glutamine > 1 mM and that glutamine had a systemic inhibitory effect on lateral root development. The negative effect of amide on root and shoot fresh weight may be probably due to that, amide decreases water uptake and relative water content as suggested by Vassilev *et al*. (1997). From other side of view, the increase and decrease in different growth parameters (figures 1 a & b) in response to asparagine or glutamine treatments may be mediated by a change in the level of naturally synthesized hormones. This conclusion is supported by Groot *et al*. (2003).

Changes in pigment contents: Biosynthesis of chlorophylls in treated french bean plant was markedly activated by low level (1 mM) and inhibited by the higher levels (3, 4 & 5 mM) of glutamine and asparagine (Fig 2 a&b). In accord with those results, Keller *et al*. (2001) demonstrated an increase in chlorophyll content and photosynthesis of vine plant by low soil nitrogen. On the other hand, Martin *et al*. (2002) observed a significant reduction in chlorophyll content in Arabidopsis seedlings grown in low nitrogen concentration. The observed progressive increases as well as the progressive decreases in pigment contents (chl a, chl b, carotenoids and total chlorophyll) *in vitro*-cultured tissues of French bean during the entire periods of experiment treated with different concentrations of either asparagine or glutamine were in good support to the growth rate (figures 1 a& b) as well as to the change in carbohydrate content (figures 3 a& b) of the same tissues. In this connection, Wettlaufer and Obendorf (1991) have reported that, treatment of soybean with glutamine or asparagine resulted in increasing fresh weight and retention of green color.

Changes in carbohydrate content: Data in figures 3 a& b showed a significant increase in glucose, sucrose, total soluble sugars and







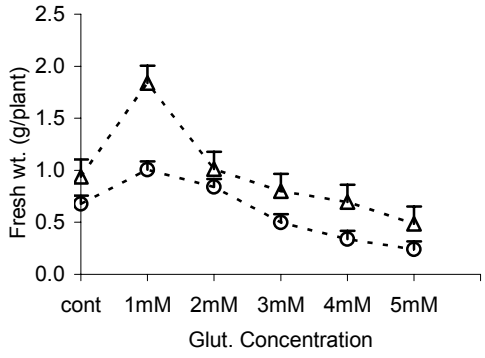
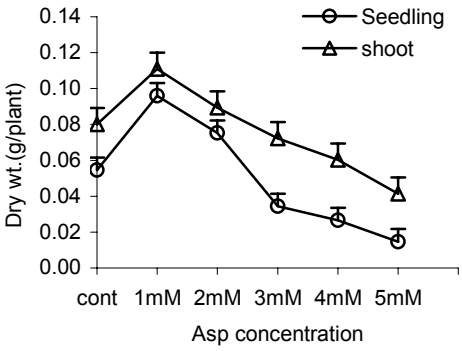
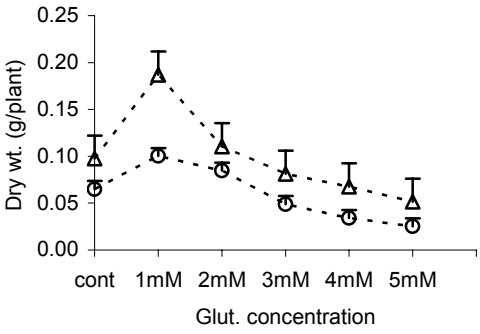
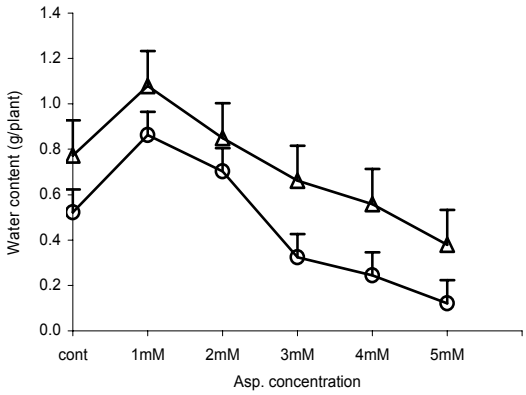


Figure. 1a: Effect of different concentrations of asparagine or glutamine on length and fresh weight of *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at P ≤ 0.05.







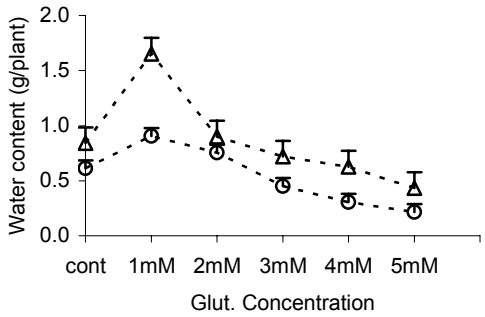


Figure. 1b: Effect of different concentrations of asparagine or glutamine on dry weight and water content of *Phaseolus vulgaris* plant at seedling and vegetative stages.

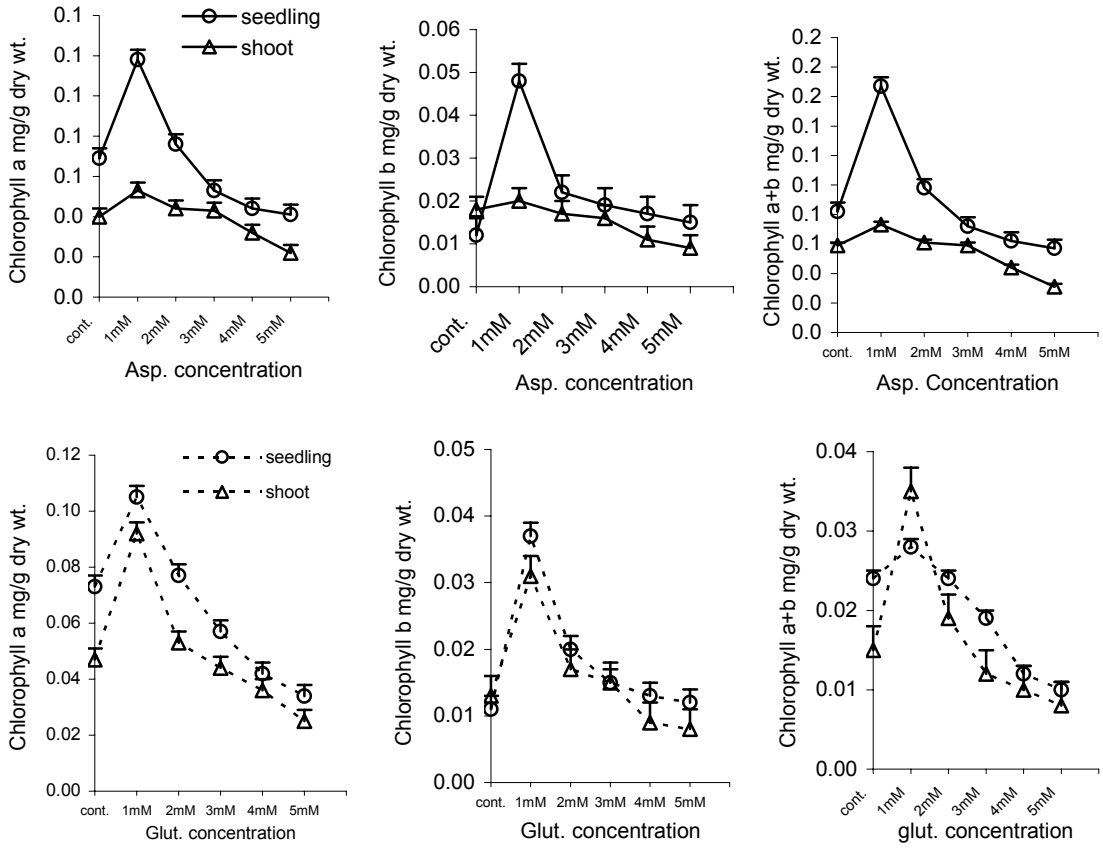


Figure. 2a: Effect of different concentrations of asparagine or glutamine on chlorophyll a, chlorophyll b and chlorophyll a+b in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at P ≤ 0.05.

total carbohydrate with 1& 2 mM asparagine or glutamine. This is in accordance with findings reported by Nakano *et al.* (1998) in rice and *Phaseolus vulgaris* when both species were grown at different nitrogen concentrations and starch and sucrose contents in both species were increased at lower nitrogen rates. Martin *et al.* (2002) also, detected slight increase in endogenous sugars in Arabidopsis seedlings in relation to low nitrogen concentration (0.1 mM). An opposite pattern of changes was shown for the different carbohydrate compounds in French bean seedlings treated with 3, 4 and 5 mM asparagine or glutamine. These results are in agreement with Druege *et al.* (2000) who found that increasing nitrogen supply resulted in substantial lower starch level and higher sucrose concentration in leaves of two *Chrysanthemum* cultivars (puma and cassa). On the other hand, Cazetta *et al.* (1999) reported that, increasing the nitrogen supply to *Zea mays* L. led to increase in starch and decrease in the level of carbon metabolites such as sucrose and reducing sugars. Schröder *et al*. (2005) stated that pyrimidines are particularly important in dividing tissues as bulding blocks for nucleic acids , but they are equally important for many biochemical processes , including sucrose and cell wall polysaccharide metabolism .

Changes in nitrogen content: In many plants asparagine and glutamine are the key intermediates in the pathway of nitrogen assimilation (Pal'ove- Balang, 2002). Treatment of French bean plants with increasing concentrations of either asparagine or glutamine induced a progressive significant increase in amide, protein and total nitrogen contents in micropropagated tissues during seedling and vegetative stages, as compared with controls. On the other hand, ammonia, peptide and total soluble nitrogen were found to decrease progressively with an increasing concentration of either asparagine or glutamine through the two stages of plant growth and development (figures 4 a & b). In this respect, Youssefi *et al.* (2000) reported

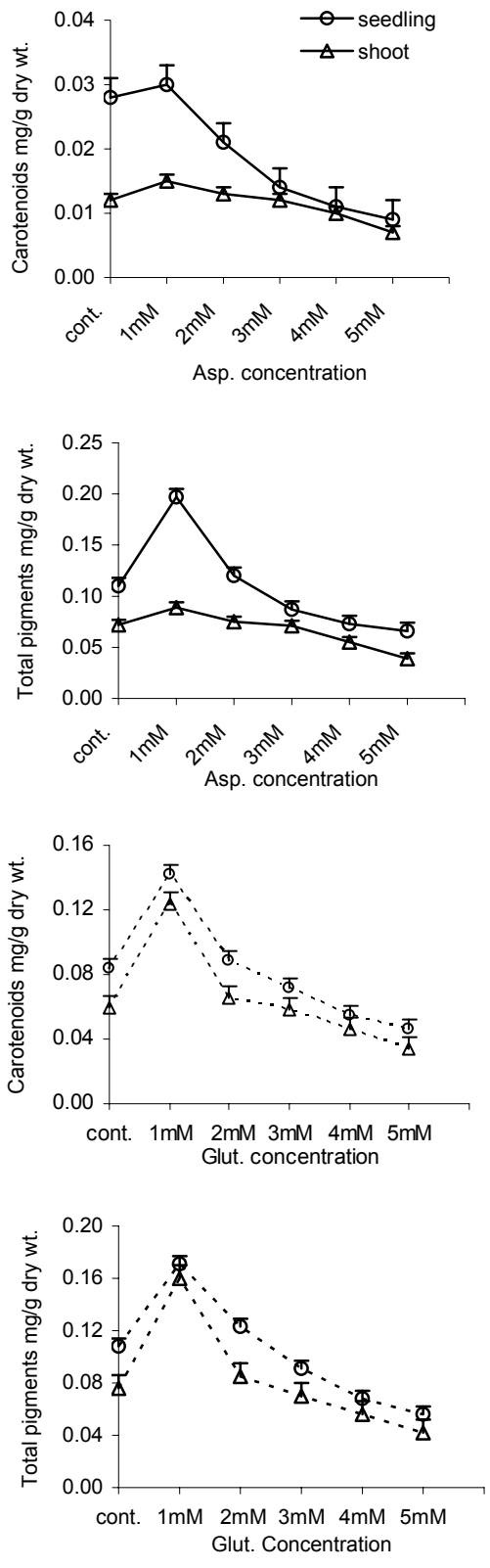


Figure. 2b: Effect of different concentration of asparagine or glutamine on carotenoids and total pigments in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = value of LSD at P ≤ 0.05.

that leaf-nitrogen concentrations were related positively to concentrations of applied amino acids (especially asparagine and glutamine). On the other hand, increasing the nitrogen supply, to *Zea mays* L. led to increase in the activity of certain enzymes, starch and the levels of nitrogen compounds (total nitrogen, soluble protein and free amino acids) and decreased the levels of carbon metabolites (sucrose and reducing sugars) in the tested plant (Cazetta *et al.* 1999).

Regarding protein content, a general significant increase in protein content in *Phaseolus* plant at seedling and vegetative stages was observed, in vitro, with increasing asparagine or glutamine concentrations. In this respect, it has been reported that the application of different nitrogen sources led to protein accumulation in plant tissue of soybean (Mosquim & Sodek ,1992) and maize (Niharika *et al.* 1998). In this context, protein synthesis which was influenced by asparagine and glutamine, suggested that amide not only effect the biosynthesis of proteins and/or amino acids but also acts as an activator of proteolytic enzymes.

Changes in ions content: All determined elements (K^+, Na^+, Mg^{++} & Ca^{++} ions) increased significantly with 1 & 2 mM asparagine or glutamine, while decreased significantly with 3, 4 and 5 mM treatments (Fig 5 a & b). In this connection, Sagi *et al.* (1998) reported that, the total cations increased in *Lolium multiflorum* with increasing nitrogen concentration in the growth medium.

The respective increase and decrease in inorganic ion contents (K^+, Na^+, Mg^{++} & Ca^{++}) at low and high concentration of asparagine or glutamine is expected to be influenced by the effect of amide-nitrogen compounds on protein synthesis as shown figure (4 b), as proteins are required to transport protons, inorganic ions and organic solutes across the plasma membrane and tonoplast at rates sufficient to meet the needs of the cells (Schroeder *et al.* 1999). In addition, multiple membrane proteins may be needed for cations uptake from soil or solution to adopt varying extracellular conditions and nutrient availability (Chrispeels *et al.* 1999). Positively charged macronutrients such as potassium (K+), calcium (Ca^{++}), and magnesium (Mg^{++}) are required in relatively large amount for plant growth and

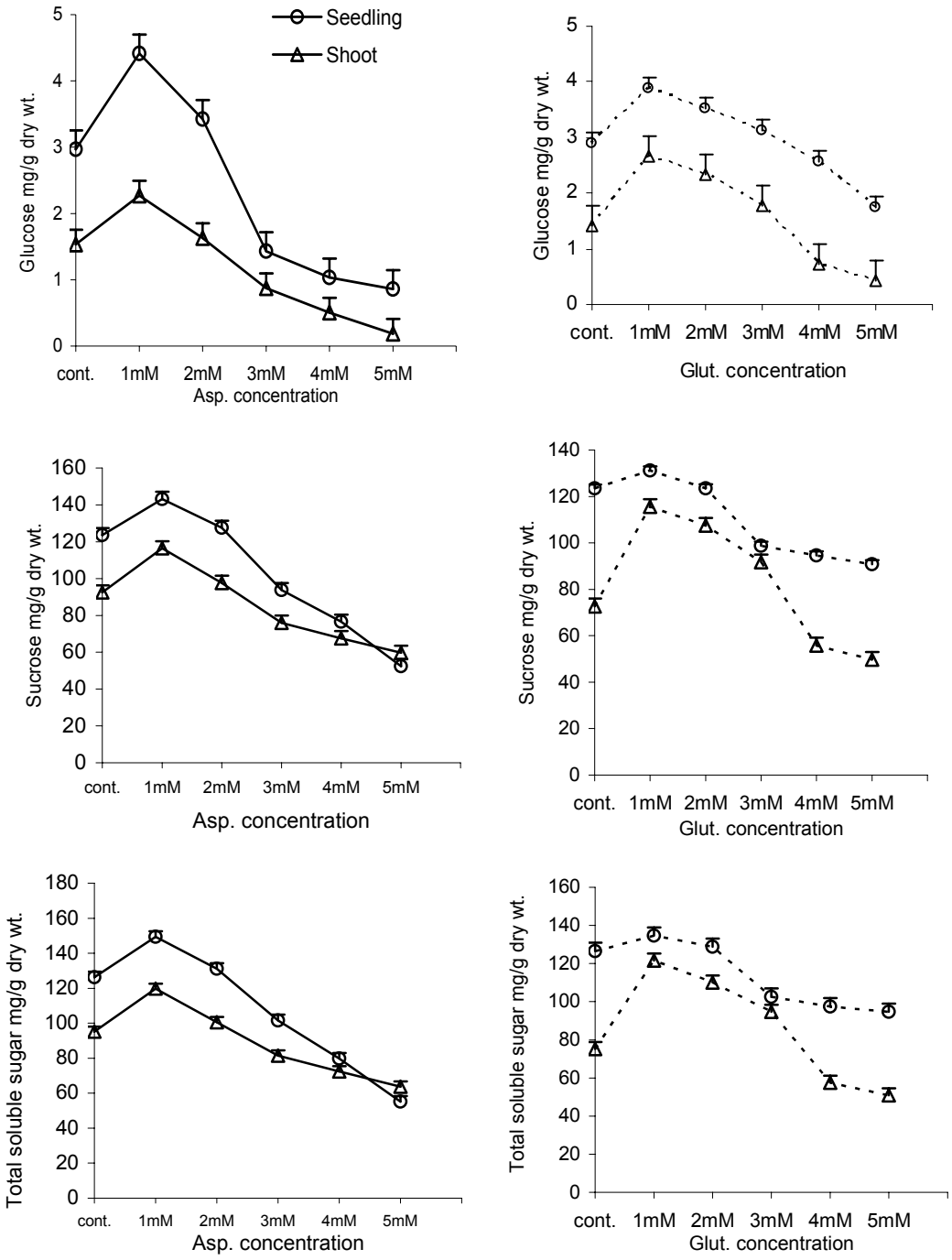


Fig. 3a: Effect of different concentrations of asparagine or glutamine on glucose, sucrose and total soluble sugar contents in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at P ≤ 0.05.

development. Additional cationic micronutrients (Fe^{++}, Mn^{++}, Zn^{++}, Cu^{++} and Ni++) play essential roles as cofactors and activators of enzymes. Thus, the above mentioned results are consistent with the results of growth parameters (1a & b) and also with pigments (2 a & b). This means asparagine and glutamine influence the absorption and transport of cations, as reported by Robinson *et al*. (1983).

Changes in growth regulators: A significant increase in growth promoters *i.e.* auxins, gibberellins and cytokinins with 1 & 2 mM

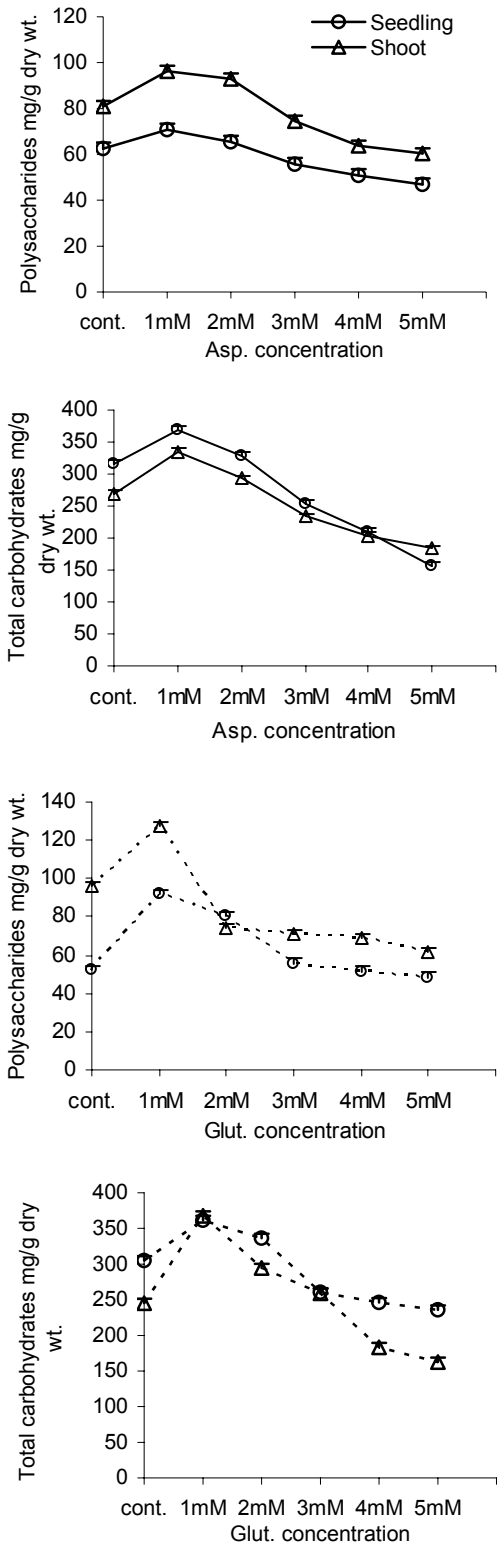


Fig.3b: Effect of different concentrations of asparagine or glutamine on polysaccharides and total soluble sugar contents in Phaseolus vulgaris plant at seedling and vegetative stages. Vertical bar = the value of LSD at P ≤ 0.05.

asparagine or glutamine while a significant decrease with higher concentrations (3, 4 and 5 mM) was observed in present study (figures 6 a & b). On the other hand ABA level significantly decreased with 1 mM and increased with 2, 3, 4 and 5 mM asparagine or glutamine. In this connection, a significant increase in the growth regulators in response to glutamine treatment has been reported in bromeliad species (Mercier and Kerbawy (1998). The promotion of growth (in response to low amide levels) occurred concurrently with increased levels of auxins, gibberellins and cytokinins and decreased ABA content, whereas opposite changes in these growth regulators were found to accompany the inhibition of growth(see figures 1 a & b).

In conclusion, the decrease in auxins as a result of amide application; particularly 5 mM might be because amide stimulates the formation of IAA-oxidase and peroxidase leading to destruction of IAA in french bean plant and/or due to decrease in IAA biosynthesis in plant tissues (Torrey, 1976). Also the noticeable decline in gibberellins of French bean plant caused by amide application may result from conversion of free active gibberellins into bound inactive gibberellins, and/or may come from the fact that, amide treatment may interfere with the metabolism of gibberellins; thus causing deactivation of gibberellins or inhibiting their biosynthesis (Ungar and Binet, 1975). On the other hand, the increase in ABA content in French bean plant may probably be due to its biosynthesis within seedlings, and /or the interference of amide with hormone metabolism by preventing the ABA catabolism (Walton, 1980).

Changes in enzymes activity: Asparagine synthetase and glutamine synthetase are key enzymes of nitrogen metabolism in higher plants (Romagni *et al.* 2000). Concerning the effect of asparagine or glutamine on enzyme activities, perusal of the data in figures 7 a & b revealed that, the activities of asparagine synthetase, glutamine synthetase, nitrate reductase and protease significantly decreased with increasing asparagine or glutamine concentrations during the two stages of plant growth. In accordance with the present results Sivasankar *et al*. (1997) has reported that the application of exogenous amides (asparagine or glutamine) to maize seedlings led to decrease in nitrate reductase

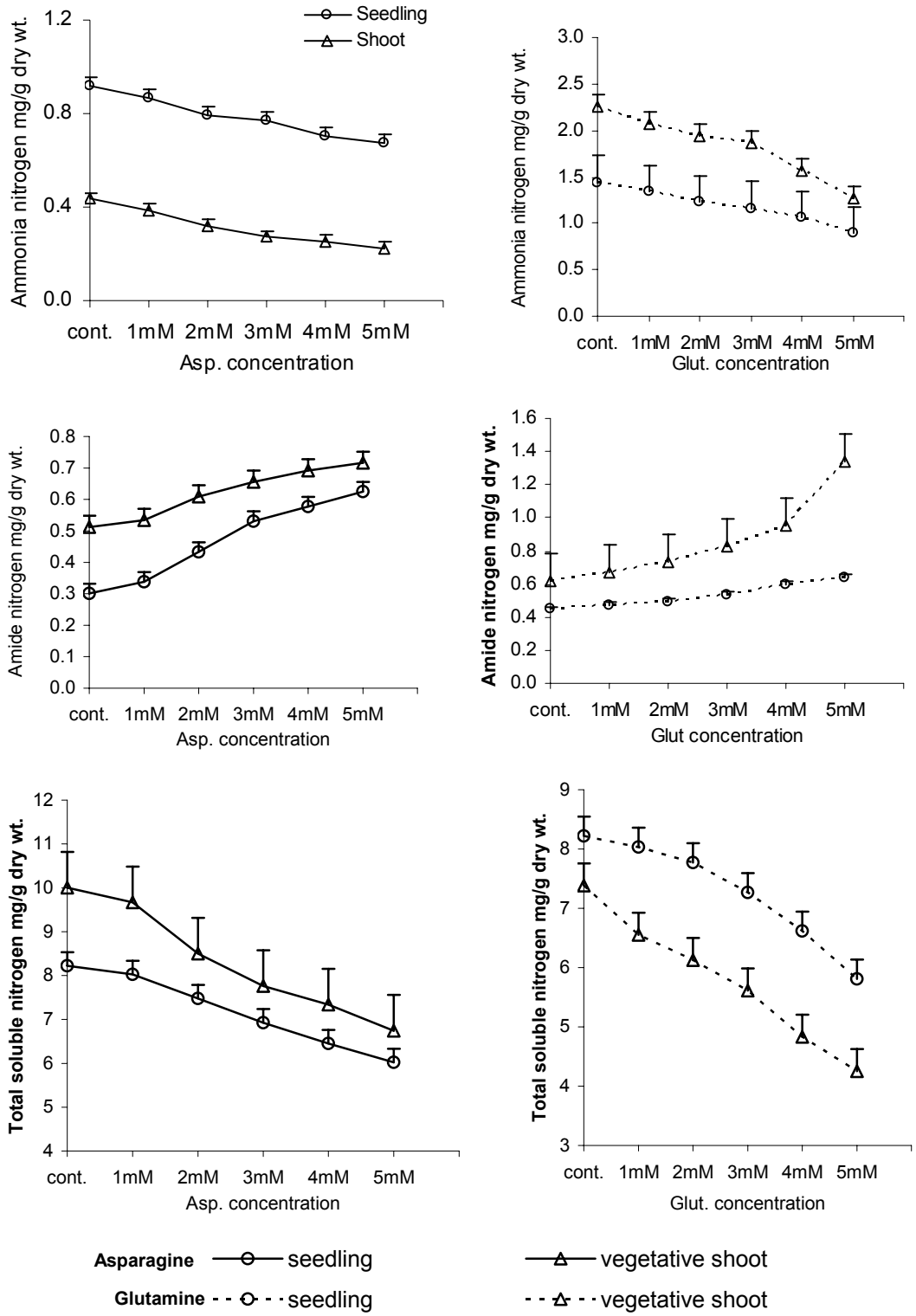


Fig. 4a: Effect of different concentrations of asparagine or glutamine on ammonia nitrogen, amide nitrogen and total soluble nitrogen in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at $P \leq 0.05$.

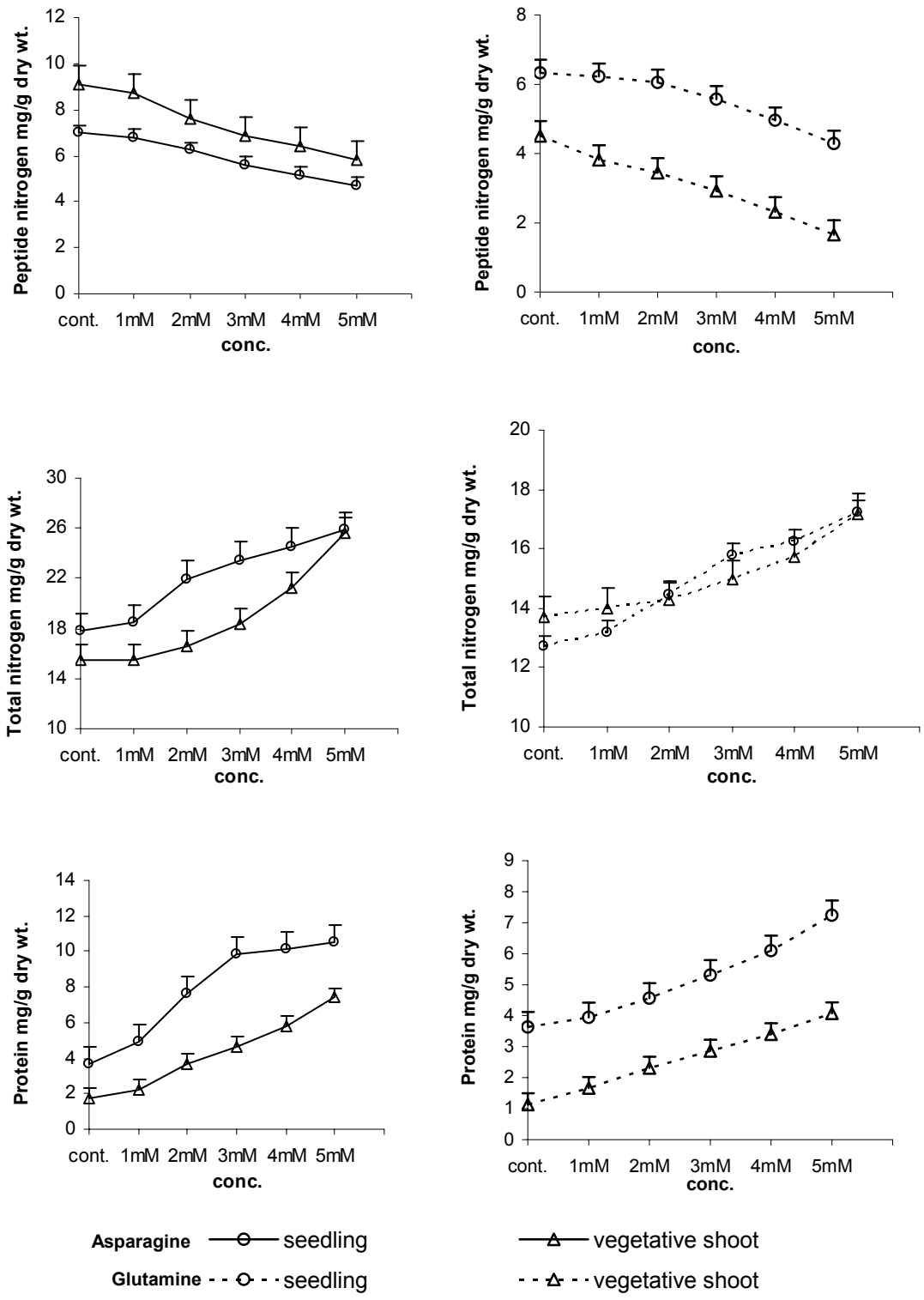


Fig. 4b: Effect of different concentrations of asparagine or glutamine on peptide nitrogen, total nitrogen and protein in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at $P \leq 0.05$.

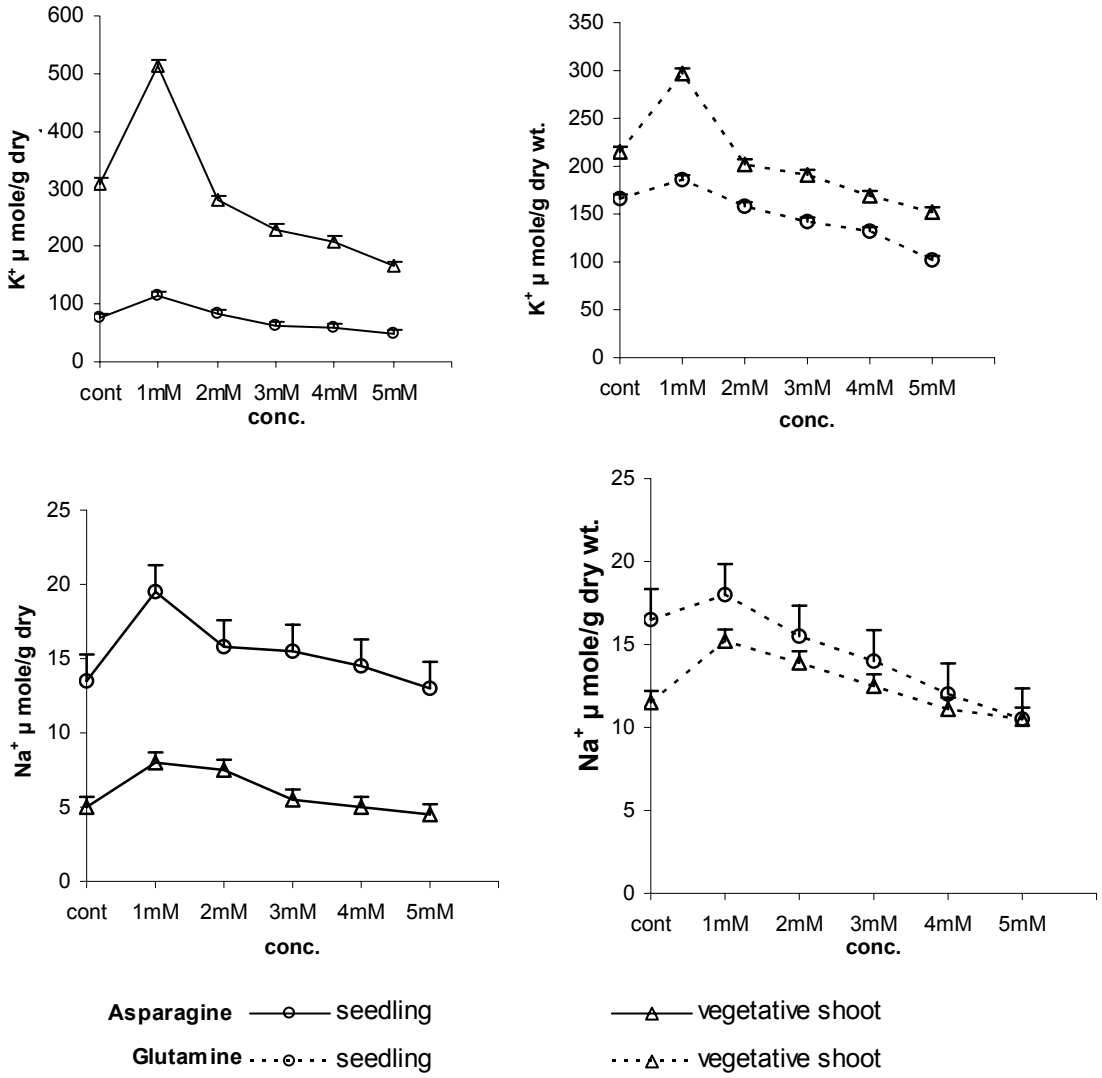


Fig. 5a: Effect of different concentrations of asparagine or glutamine on K^+ and Na^+ ions in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at $P \leq 0.05$.

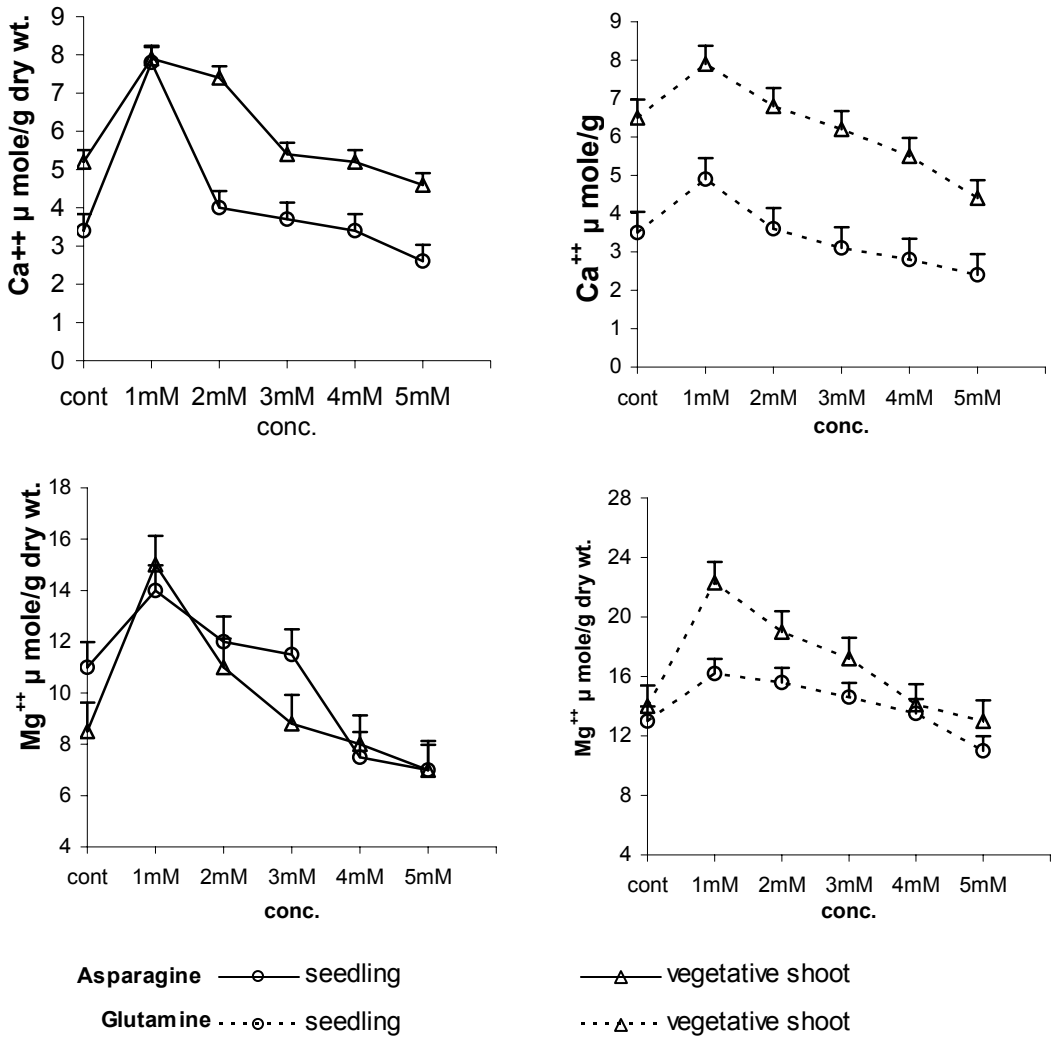


Fig. 5b: Effect of different concentrations of asparagine or glutamine on Ca^{++} and Mg^{++} ions in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at $P \leq 0.05$.

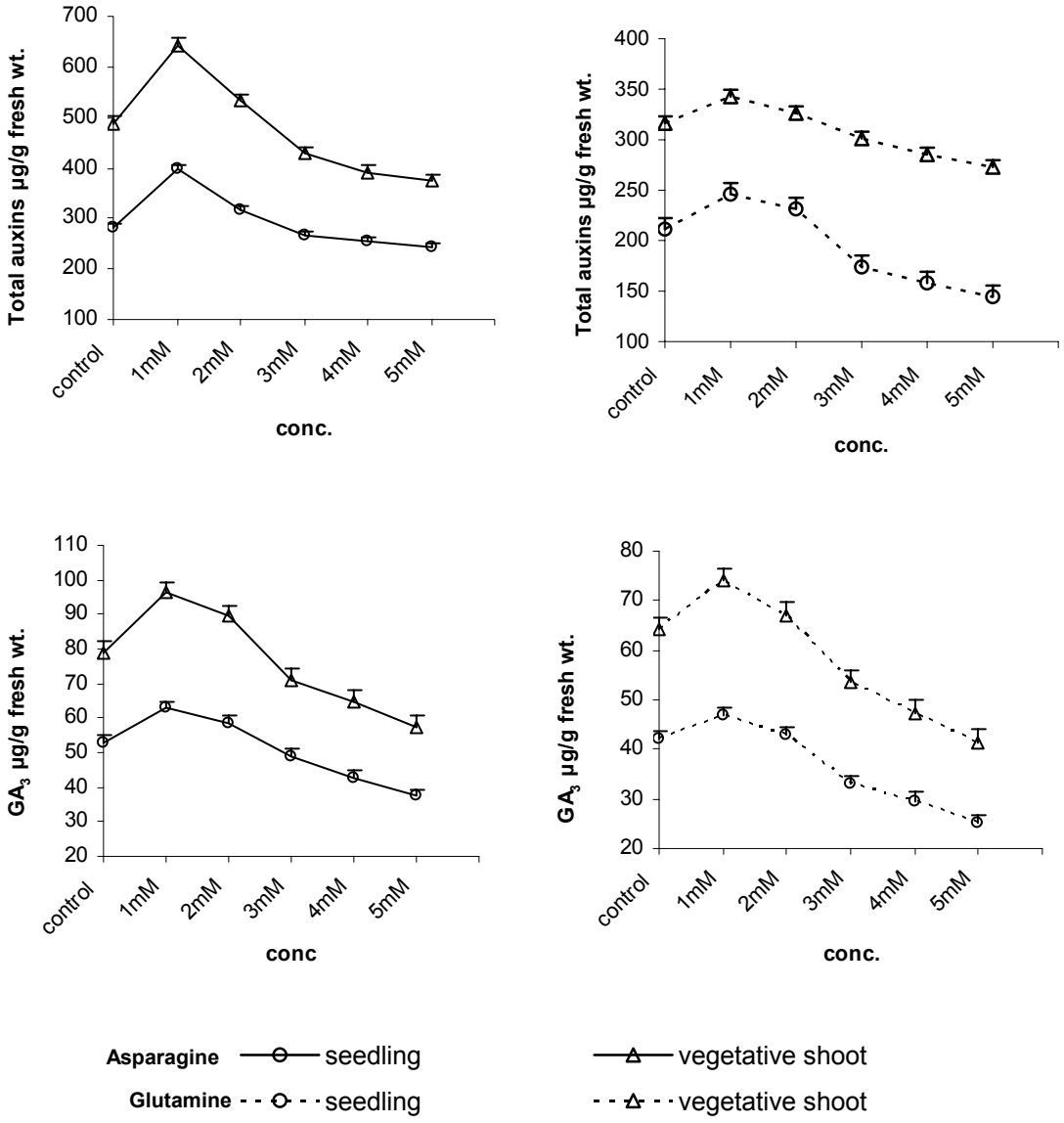


Fig. 6a: Effect of different concentrations of asparagine or glutamine on auxins and GA3 contents in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at P ≤ 0.05.

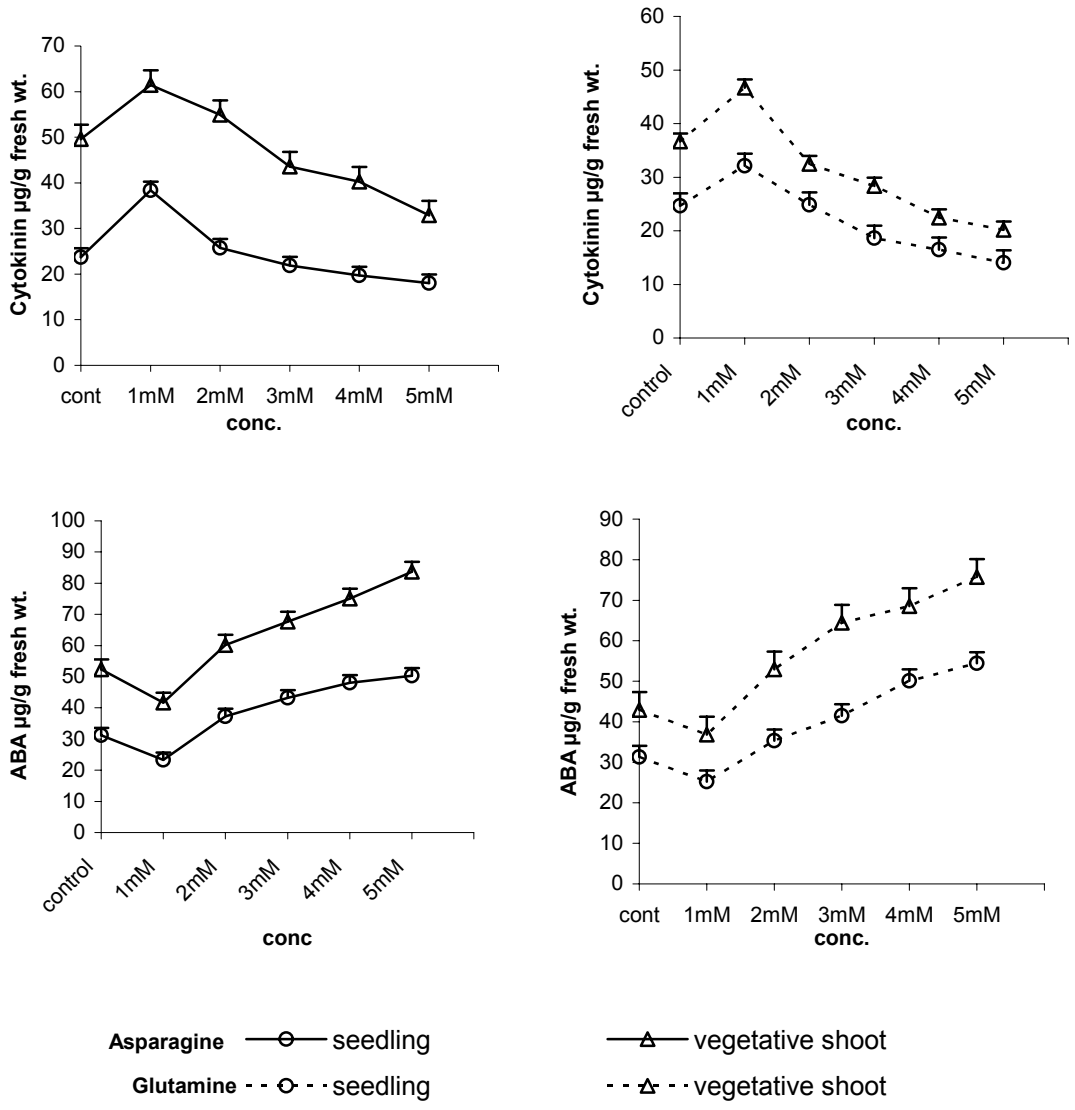


Fig. 6b: Effect of different concentrations of asparagine or glutamine on cytokinin and ABA contents in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at P ≤ 0.05.

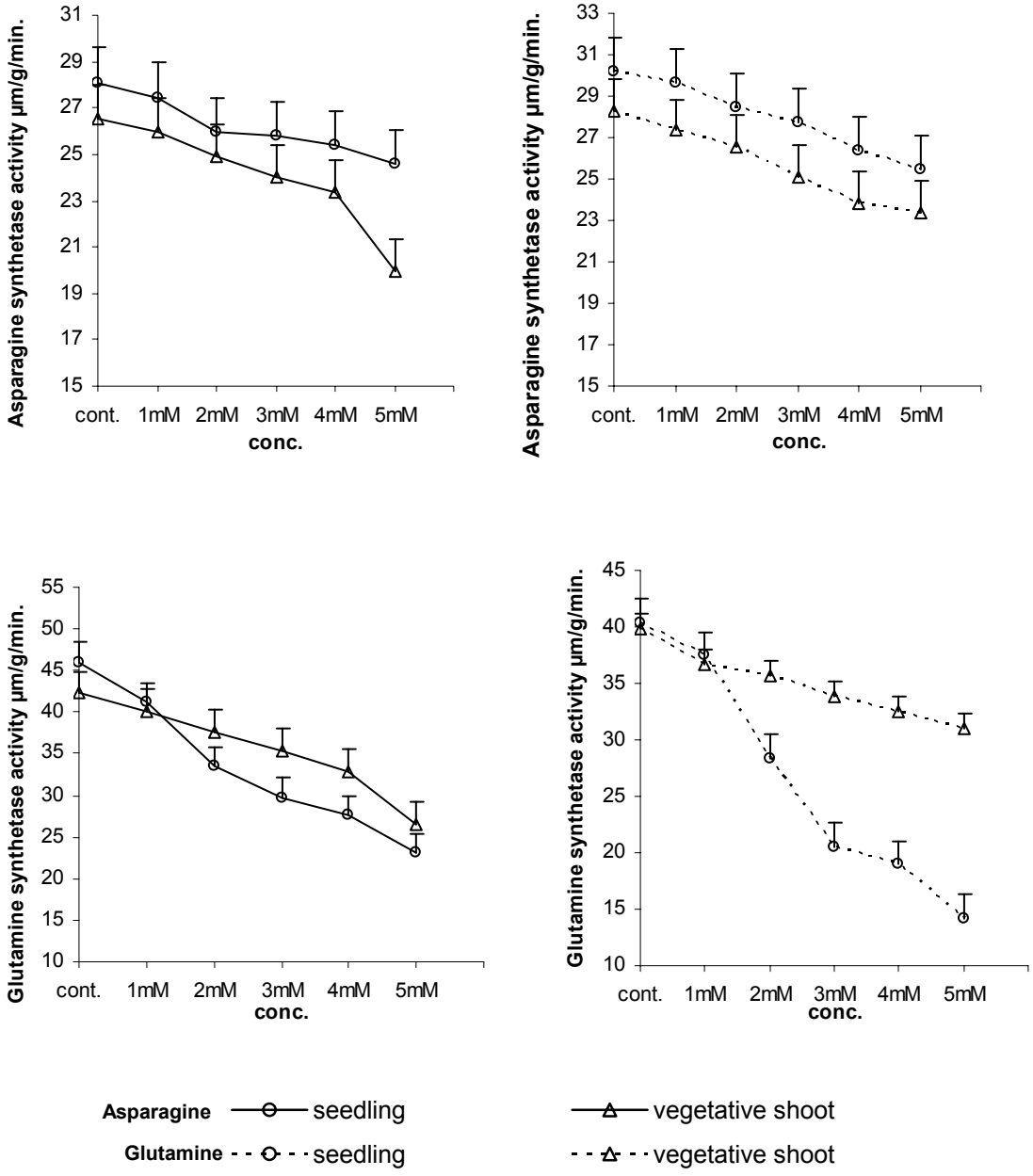


Fig. 7a: Effect of different concentrations of asparagine or glutamine on the activity of asparagine synthetase and glutamine synthetase in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at $P \leq 0.05$.

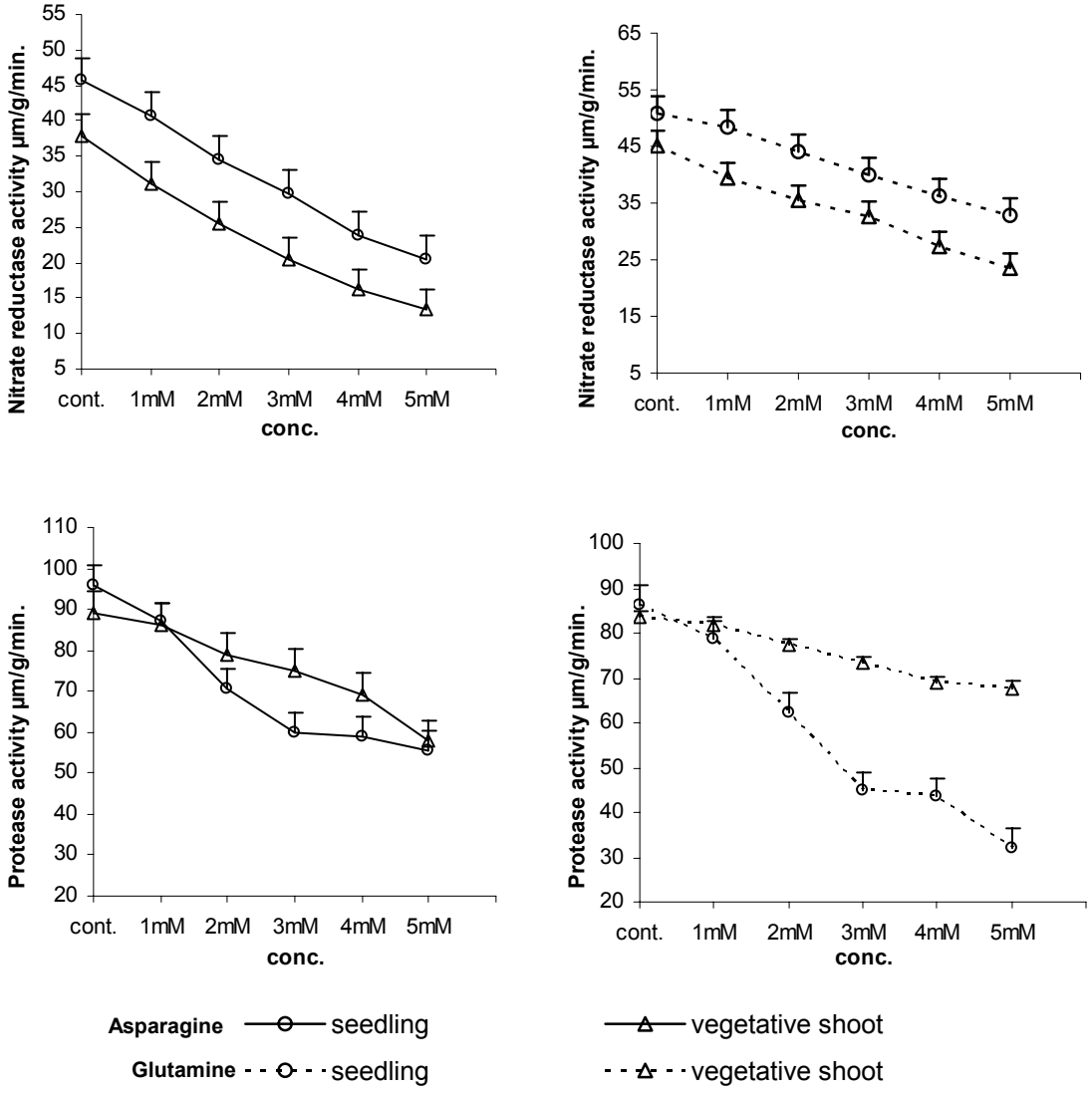


Fig. 7b: Effect of different concentrations of asparagine or glutamine on the activity of nitrate reductase and protease in *Phaseolus vulgaris* plant at seedling and vegetative stages. Vertical bar = the value of LSD at P ≤ 0.05.

activity. The predominant role of glutamine synthetase and glutamate synthase in the assimilation of nitrogen has been reported in soybean (Schubert, 1986). On the other hand, Pal'ove-Balang and Mistrik (2001) examined nitrate reductase activity in maize, after treatment with two different concentrations (1 and 10 mM) of glutamine or asparagine and found that, the low concentration (1 mM) showed no effect on nitrate reductase activity, and the higher concentration (10 mM) stimulate the enzyme. In addition, Majerowicz *et al*. (2000) using *Catasetum fimbriatum* and Sagi *et al.* (1998) using *Lolium multiflorum* stated that glutamine synthetase activity was slightly affected by nitrogen source.

Furthermore, glutamine supplementation reduced the glutamine synthetase activity in *Asparagus officinalis* L. (Seelye *et al.* 1995). It has also been reported that glutamine synthetase and nitrate reductase gradually increased with increasing nitrogen treatment in root and leaves of sugar beet (Cai-Feng *et al*. 2002). In conclusion, the changes in the different enzyme activities by application of asparagine and glutamine in french bean plants may be attributed to the amide action on the biosynthesis of enzyme protein, enzyme activation and/or membrane permeability (Storey, 1978).

It can be concluded that, changes in carbohydrates content, pigment content, nitrogen content, protein content, ion content, growth regulators content and enzymes activity and growth response in French bean under *in-vitro* conditions appeared to be a function of metabolic disorders as influenced by increasing concentrations of the used amide-nitrogen compounds (asparagine and glutamine).

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