

Performance of Faba Bean under Calcium and Gibberellic Acid Application

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ABSTRACT

The main objective of the present studies was to determine the best dose of calcium (Ca^{+2}) in Experiment 1, and in Experiment 2 aimed to test whether the growth and photosynthetic pigments of faba bean (*Vicia faba* L.) cv. 'TARA' could be enhanced by inclusion of GA_3 in the basal treatments containing Ca^{+2} . In Experiment 1, application of Ca^{+2} significantly enhanced almost all growth and photosynthetic pigments and enzyme carbonic anhydrase (CA) activity. Among the treatments, 60 mM Ca^{+2} proved best. Treatment with 60 mM of Ca^{+2} significantly increased plant height, shoot fresh weight (FW), shoot dry weight (DW), root length, root FW, root DW and root number, relative water content and chlorophyll (Chl) *a*, *b*, total Chl, anthocyanin and CA activity compared to the control. In Experiment 2, application of Ca^{+2} with GA_3 significantly enhanced almost all growth parameters. Among the treatments, 20 mM Ca^{+2} with 10^{-6} M GA_3 gave the maximum value for almost all parameters studied compared to the control as well as the application of GA_3 (10^{-6}) alone. The application of Ca^{+2} along with GA_3 more efficiently ameliorates the growth and photosynthetic capacity of faba bean than Ca^{+2} alone.

Keywords: carbonic anhydrase, growth regulator, photosynthetic pigments, plant growth, *Vicia faba*

INTRODUCTION

Faba bean (*Vicia faba* L.) is a high protein crop grown in Europe, Africa and Asia, used to feed both animals and humans. Faba bean is mainly used as human food in developing countries and as animal feed, mainly for pigs, horses, poultry and pigeons in industrialized countries. It can be used as a vegetable, green or dried, fresh or canned. It is a common breakfast food in the Middle East, Mediterranean region, China and Ethiopia (Bond *et al.* 1985). Therefore, it has an increasing importance for human as well as animal food in the future. Legumes play a critical role in natural ecosystems, agriculture, and agroforestry, where their ability to fix N in symbiosis makes them excellent colonizers of low-N environments, and economic and environmentally friendly crop, pasture, and tree species. Legume yields unfortunately continue to lag behind those of cereals. A serious problem to the production of faba bean caused by lack of knowledge of the precise dose of fertilizers recommended by the Agriculture Department for a particular cultivar and region.

Calcium (Ca^{+2}) is, an essential plant nutrient, a critical part of the cell wall that produces strong structural rigidity by forming cross-links within the pectin polysaccharide matrix. With rapid plant growth, the structural integrity of stems that hold flowers and fruit, as well as the quality of the fruit produced, is strongly coupled to Ca^{+2} availability. Ca^{+2} is an obligate intracellular messenger coordinating responses to numerous developmental cues and environmental challenges (Easterwood 2002; Marschner 2002; White and Broadley 2003). The function of GA as a hormone in regulating plant growth was known as early as the 1950s (Vlitos and Meudt 1957; Brian 2008). Gibberellins are associated with various plant growth and development processes such as seed germination, stem and hypocotyl elongation, leaf expansion, floral initiation, floral organ development, fruit development, sex determination and induction of some hydrolytic enzymes in the aleurone of cereal grains (Silverstone and Sun 2000; Asahina *et al.* 2002; Fleet and Sun

2005). GA_3 also plays an important role in alleviating abiotic stress such salinity and induction of nitrogen use-efficiency (Siddiqui *et al.* 2008; Khan *et al.* 2010). Khan *et al.* (2010) reported that GA_3 increased plant height, number of branches, number of leaves, leaf area, fresh and dry weights of linseed.

It was, therefore, decided to undertake two greenhouse pot experiments on newly released cultivar (TARA) of faba bean. The first experiment was planned on the cultivar of faba bean to select the best dose of Ca^{+2} on the basis of morphological and physiological attributes. Second experiment was aimed to test whether the morphological and physiological characteristics of the 'TARA' cultivar of faba bean could be enhanced by inclusion of GA_3 in the basal treatments containing Ca^{+2} .

MATERIALS AND METHODS

To meet the objectives mentioned in introduction, the responses of faba bean to Ca^{+2} and GA_3 applications were studied by conducting two greenhouse pot experiments during the '2010-2011 at the Department of Botany and Microbiology, King Saud University, Riyadh, KSA. Finally, 52 pots (6 inch diameter) were arranged in a simple randomized design with a single factor and four replicates and each pot filled equally with perlite and watered with double distilled water (DDW) lightly before sowing to maintain proper moisture content in the surface of the perlite. Authentic seeds of faba bean 'TARA' were obtained from the local market of Riyadh. Before sowing, the viability of seeds of cultivar was tested and they were surface sterilized with ethyl alcohol then vigorously rinsed with DDW. Five seeds in each pot were sown (2 to 2.5 cm deep) in perlite-filled pots supplied with Raukura's nutrient Solution (Smith *et al.* 1983). In Experiment 1, six graded levels of Ca^{+2} , viz. Ca_0 , Ca_{20} , Ca_{40} , Ca_{60} , Ca_{80} and Ca_{100} mM were applied basally. In Experiment 2, the treatments also included Ca_0 mM + GA_3 0 M, Ca_0 mM + GA_3 10^{-6} M, Ca_{20} mM + GA_3 10^{-6} M, Ca_{40} mM + GA_3 10^{-6} M, Ca_{60} mM + GA_3 10^{-6} M, Ca_{80} mM + GA_3 10^{-6} M, and Ca_{100} mM + GA_3 10^{-6} M. The source of Ca^{+2} was calcium chloride. Each pot was given 200 mL of nutrient solution at every week. When

the plants were at the stage of 2–3 true leaves, Ca⁺² solution was added to the pots with experimental faba bean plants. The plots received irrigations (300 mL DDW in each pot) at every 3 days. The sampling was done at 45 days after sowing. To study the growth performance (plant height plant⁻¹, shoot fresh weight (FW) plant⁻¹, shoot dry weight (DW) plant⁻¹, root length plant, root fresh weight plant⁻¹, root dry weight plant⁻¹, root number plant⁻¹, leaf number plant⁻¹, relative water content (RWC)) and chlorophyll (Chl) *a*, *b*, total Chl, anthocyanin and CA activity of the *V. faba* plants were sampled randomly from each pot of Experiments 1 and 2.

The plant height and root length of plant were measured by using a meter scale after removal from the pots. The plants were then placed in an oven run at 60°C for 48 h. These dried plants were weighed to record the plant dry weight.

The RWC was expressed that in percentage the water content at a given time and tissue as related to the water content at full turgor (Slatyer 1967). Four leaves were collected from plants of each replicates of the treatments and weighed for fresh weight. Leaf pieces were kept in Petri dishes having de-ionized water and left for overnight and kept away the samples from physiological activity by physical inhibition of growth and respiration (placed in fridge at darkness). After 24 hrs, leaves were blotted for removing any free surface moisture and reweighed for turgid fresh weight (TFW). Leaf sample were kept in oven at 60°C for 48 h and reweighed for DW and finally calculated relative water content. The relative water content was calculated using the following formula given by González and González-Vilar (2001):

$$\text{RWC (\%)} = [(\text{FW} - \text{DW}) / (\text{TFW} - \text{DW})] \times 100.$$

The activity of (CA: EC 4.2.1.1) activity was determined by the method of Dwivedi and Randhawa (1974). The leaf samples were cut into small pieces and suspended in cystein hydrochloride solution. The samples were incubated at 40°C for 20 min. The pieces were blotted and transferred to the test tubes containing phosphate buffer (pH 6.8), followed by the addition of alkaline bicar-

bonate solution and bromothymol blue indicator. The test tubes were incubated at 50°C for 20 min. After the addition of 0.2 mL of methyl red indicator, the reaction mixture was titrated against 0.05 N HCl. The results were expressed as [$\mu\text{mol} (\text{CO}_2) \text{kg}^{-1} (\text{f.m.}) \text{s}^{-1}$].

Chlorophyll (Chl) was extracted from freshly leaves of experimental plants using the DMSO method based on Barnes et al. (1992). Chl absorption in the extract was measured using UV-VIS spectrophotometer. Content of the Chl was calculated from the following formulae taken from Barnes et al. (1992):

$$\text{Chl } a = 14:85A_{664.9} - 5:14A_{648.2}$$

$$\text{Chl } b = 25:48A_{648.2} - 7:36A_{664.9}.$$

Statistical analysis

Each experimental pot was treated as one replicate and all the treatments were repeated four times. The data were analysed statistically with SPSS-11 statistical software (SPSS Inc., Chicago, IL, USA). Mean was statistically compared by Duncan's multiple range test (DMRT) at $P > 0.05\%$.

RESULTS

The data (**Table 1**) reveal that the growth characteristics of faba bean increased with increasing levels of Ca⁺² up to 60 mM in Experiment 1. However, treatment 20 mM of Ca⁺², showing parity with treatment 40, 60 and 100 mM of Ca⁺², gave maximum value for root length. Application of 60 mM of Ca⁺² enhanced maximum plant height, shoot FW, shoot DW, root FW, root DW and RWC over the respective controls. Moreover, the effect of 60 mM of Ca⁺² was at par with 20 and 40 mM of Ca⁺² for plant height and 40 mM of Ca⁺² for shoot FW. Application of 40, 60 and 80 mM of Ca⁺² showed equal effect for root number. However, in Experiment 2, inclusion of GA₃10⁻⁶ M in basal treatments containing Ca⁺² enhanced significantly almost growth attributes

Table 1 Effect of Ca⁺² and GA₃ on growth performance of faba bean. Mean of four replicates with S.E. and the same letter do not differ statistically at $P < 0.05$ (Duncan's Multiple Range Test).

Treatments	Plant height (cm)	Shoot FW (g)	Shoot DW (g)	Root length (cm)
Experiment 1				
Ca ₀	21.10 ± 3.19 c	3.47 ± 0.13 b	0.30 ± 0.03 d	16.50 ± 1.80 b
Ca ₂₀	30.73 ± 2.34 ab	4.23 ± 0.61 b	0.31 ± 0.01 cd	25.00 ± 3.33 a
Ca ₄₀	31.38 ± 2.66 ab	5.17 ± 0.43 ab	0.39 ± 0.04 b	20.83 ± 1.17 ab
Ca ₆₀	37.75 ± 1.38 a	6.83 ± 0.97 a	0.49 ± 0.02 a	19.67 ± 2.91 ab
Ca ₈₀	28.00 ± 1.95 b	4.73 ± 0.79 b	0.39 ± 0.02 bc	22.83 ± 1.30 ab
Ca ₁₀₀	29.63 ± 1.49 b	4.97 ± 0.32 ab	0.39 ± 0.01 bc	18.83 ± 0.60 ab
Experiment 2				
Ca ₀ + GA ₃ 0	20.00 ± 1.59 d	3.40 ± 0.15 d	0.23 ± 0.01 d	19.83 ± 1.17 bc
Ca ₂₀ + GA ₃ 10 ⁻⁶	45.75 ± 1.65 a	7.77 ± 0.61 a	0.54 ± 0.03 a	28.17 ± 2.91 a
Ca ₄₀ + GA ₃ 10 ⁻⁶	35.00 ± 1.22 b	6.17 ± 0.62 b	0.45 ± 0.02 b	22.00 ± 2.08 abc
Ca ₆₀ + GA ₃ 10 ⁻⁶	31.38 ± 1.25 b	5.23 ± 0.46 bc	0.38 ± 0.03 bc	19.00 ± 1.61 bc
Ca ₈₀ + GA ₃ 10 ⁻⁶	25.50 ± 0.96 c	4.57 ± 0.30 cd	0.34 ± 0.02 c	21.33 ± 3.92 abc
Ca ₁₀₀ + GA ₃ 10 ⁻⁶	22.00 ± 1.24 cd	4.45 ± 0.24 cd	0.25 ± 0.02 d	17.00 ± 1.89 c

Table 2 Effect of Ca⁺² and GA₃ on growth performance of faba bean. Mean of four replicate with S.E. and the same letter do not differ statistically at $P < 0.05$ (Duncan's Multiple Range Test).

Treatments	Root FW (cm)	Root DW (g)	Root number	Relative water (%)
Experiment 1				
Ca ₀	3.85 ± 0.56 ab	0.18 ± 0.01 c	19.67 ± 6.66 b	52.15 ± 2.16 d
Ca ₂₀	3.43 ± 0.10 ab	0.24 ± 0.01 ab	32.67 ± 4.04 ab	59.78 ± 2.54 c
Ca ₄₀	4.13 ± 0.41 ab	0.21 ± 0.02 abc	39.33 ± 13.58 a	63.93 ± 1.94 bc
Ca ₆₀	4.70 ± 0.10 a	0.25 ± 0.02 a	49.00 ± 8.89 a	77.27 ± 0.61 a
Ca ₈₀	4.01 ± 0.15 ab	0.20 ± 0.01 bc	44.67 ± 9.29 a	67.08 ± 1.09 b
Ca ₁₀₀	2.97 ± 0.61 b	0.20 ± 0.00 bc	32.00 ± 14.00 ab	61.11 ± 2.16 bc
Experiment 2				
Ca ₀ + GA ₃ 0	3.44 ± 0.30 c	0.17 ± 0.04 c	27.00 ± 1.53 c	57.82 ± 2.67 d
Ca ₂₀ + GA ₃ 10 ⁻⁶	5.30 ± 0.69 a	0.29 ± 0.04 a	54.33 ± 5.81 a	79.27 ± 1.52 a
Ca ₄₀ + GA ₃ 10 ⁻⁶	5.12 ± 0.44 ab	0.25 ± 0.02 ab	42.33 ± 4.81 ab	66.27 ± 0.55 bc
Ca ₆₀ + GA ₃ 10 ⁻⁶	3.54 ± 0.47 bc	0.19 ± 0.00 bc	39.67 ± 6.94 bc	68.74 ± 1.96 b
Ca ₈₀ + GA ₃ 10 ⁻⁶	3.38 ± 0.24 bc	0.23 ± 0.02 abc	36.67 ± 2.60 bc	64.45 ± 1.44 bc
Ca ₁₀₀ + GA ₃ 10 ⁻⁶	4.09 ± 0.43 abc	0.20 ± 0.01 bc	36.00 ± 2.08 bc	60.67 ± 1.45 cd

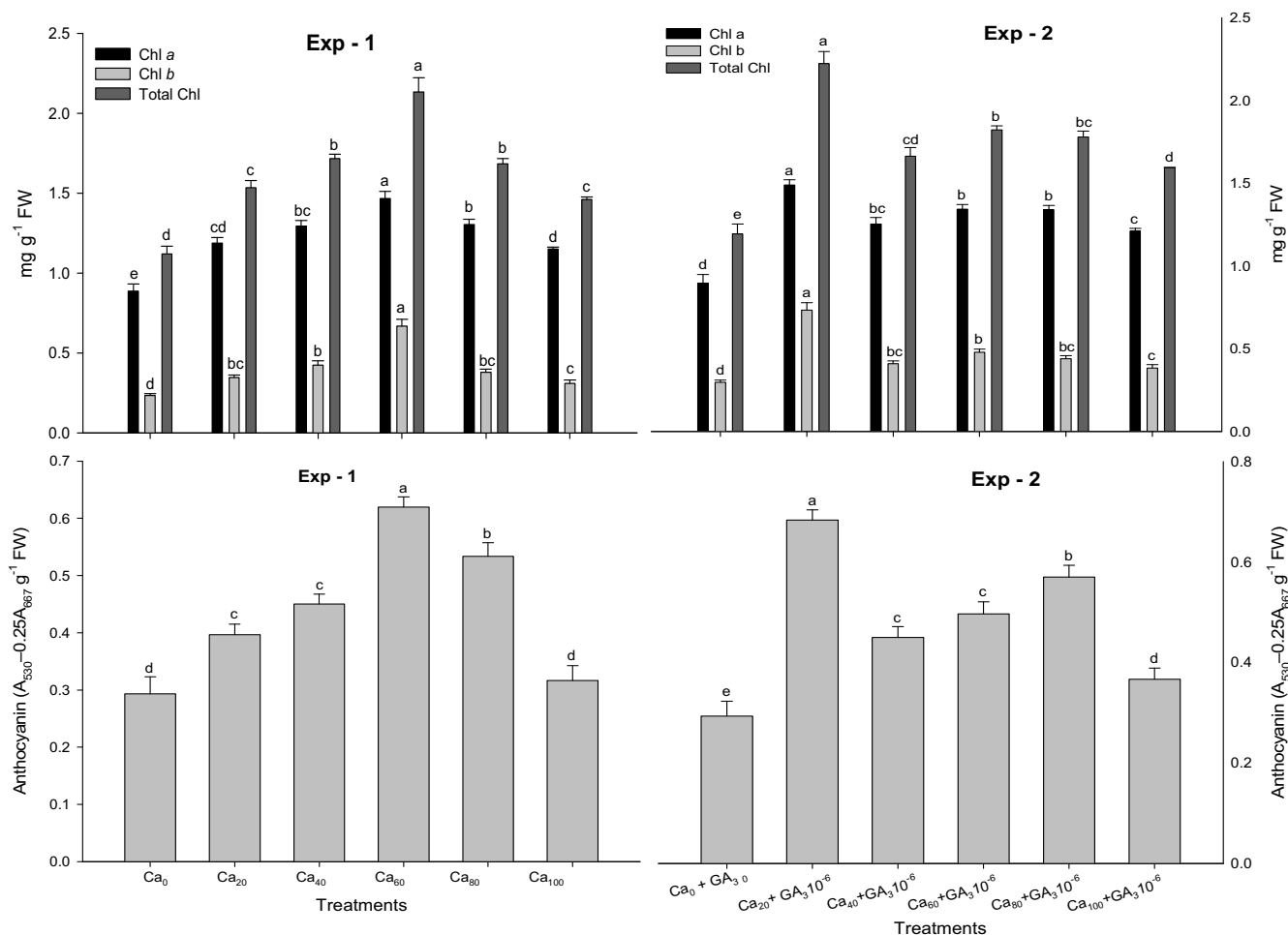


Fig. 1 Effect of Ca^{+2} and GA_3 on physiological performance of faba bean. Bars followed by the same letter do not differ statistically at $P < 0.05$ (Duncan's Multiple Range Test). Average of four determinations are presented with bars indicating S.E.

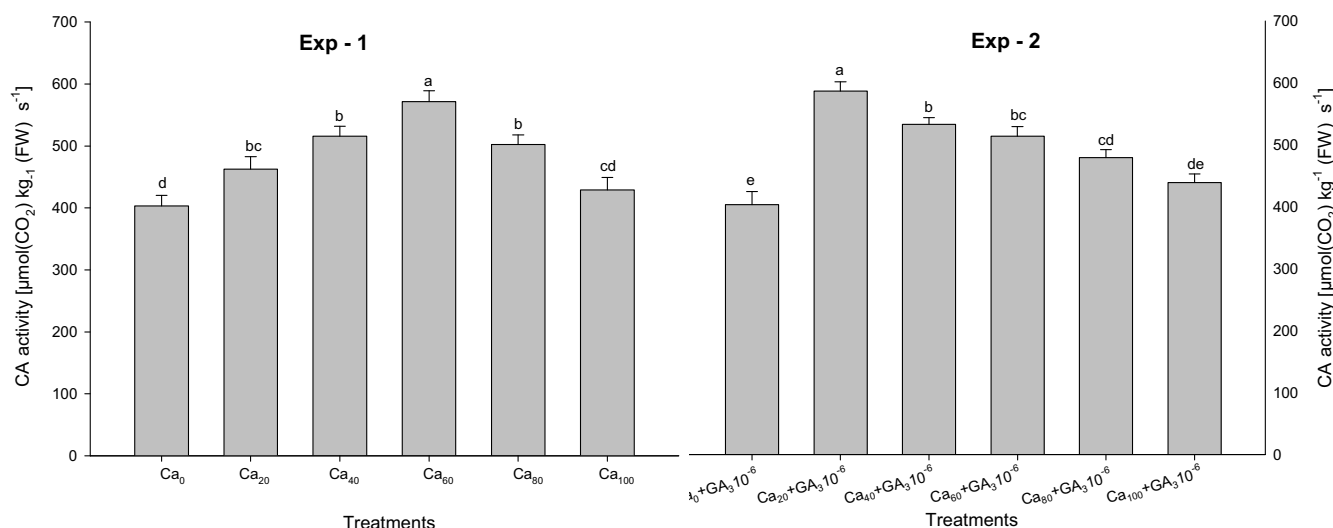


Fig. 2 Effect of Ca^{+2} and GA_3 on physiological performance of faba bean. Bars followed by the same letter do not differ statistically at $P < 0.05$ (Duncan's Multiple Range Test). Average of four determinations are presented with bars indicating S.E.

of faba bean (**Table 1**). Plant height, shoot FW, shoot DW, root length, root FW, root DW, root number and RWC were recorded maximum at 20 mM of Ca^{+2} and GA_3 10^{-6} M, compared to their respective controls and other treatments. However, 20 mM of Ca^{+2} and GA_3 10^{-6} M was followed by 40 mM of Ca^{+2} and GA_3 10^{-6} M for root length, root FW, root DW and root number.

The data presented in **Fig. 1** reveals that Chl *a*, *b*, total Chl, anthocyanin and CA activity enhanced significantly with increasing levels of Ca^{+2} up to 60 mM in Experiment 1. Treatment of 60 mM of Ca^{+2} proved best by giving maxi-

imum values for these photosynthetic pigments as compared to their respective controls. However, in Experiment 2, application of GA_3 10^{-6} M with Ca^{+2} was found more effective in enhancement of Chl *a*, *b*, total Chl, anthocyanin and CA activity than Experiment 1 (**Fig. 1**). Among the treatments, 20 mM of Ca^{+2} and GA_3 10^{-6} M had maximum enhancing effect for Chl *a*, *b*, total Chl, anthocyanin and CA activity over the respective controls.

DISCUSSION

An observed improvement over the no-nutrient control in plant height, shoot FW, shoot DW, root length, root FW, root DW, root and leaf number plant⁻¹, RWC, photosynthetic pigments (Chl *a*, *b*, total Chl and anthocyanin) and activity of CA was observed due to the application of Ca⁺² and GA₃ (Tables 1, 2; Figs. 1, 2). The enhancing effect of the application of Ca⁺² can be explained on the basis of its roles. It may be added that Ca⁺² stabilizes cell membranes by connecting various proteins and lipids at membrane surfaces, influences the pH of cells and prevents solute leakage from cytoplasm and increase shoot elongation (Tamura *et al.* 2001; Hirschi 2004) and acts as a regulator of many physiological and biochemical processes (Bush 1995). The light interacts with Ca⁺² directly in the pathway of Chl biosynthesis (Lechowski and Białczyk 1993). Nayek *et al.* (1983) reported that Ca⁺² improved the water status of the plants most prominently at the vegetative stage. Furthermore, in Experiment 2, inclusion of GA₃ (10⁻⁶ M) in Ca⁺² treatments was found more efficient for the enhancement of growth parameters, photosynthetic pigments and CA activity of faba bean. Combined application of 20 mM of Ca⁺² and GA₃ proved best as compared to other treatments (Tables 1, 2; Fig. 1). It might be GA₃ increased Ca⁺²-use efficiency in plants and other nutrients (Siddiqui *et al.* 2008). The increase in plant growth as a response to Ca⁺² and GA₃ occurs as a consequence of cell elongation and cell division (Hirschi 2004; Tanimoto 2005). It is interesting that application of Ca⁺² with GA₃ improved the root growth; it might be due to the elusive role of Ca⁺² as a second messenger in plant cell growth and development (Shabala *et al.* 2003; Hepler 2005). Ca⁺² increases ammonium, potassium and phosphorus absorption, stimulates photosynthesis, carboxylation efficiency (CE), ribulose 1,5-biphosphate carboxylase activity, chlorophyll content, and increases the size of sellable plant parts (Lechowski and Białczyk 1993; Fenn *et al.* 1995; Liang *et al.* 2009). Siddiqui *et al.* (2008) and Khan *et al.* (2010) in mustard demonstrated that GA₃ enhanced the CA activity, the enzyme which catalyzes the reversible hydration of CO₂ to HCO₃⁻. GA₃ promotes DNA, RNA and protein synthesis (Broughton and McComb 1971; Pain and Dutta 1977; Mozer 1980) and ribose and polyribosome multiplication (Evins and Varner (1972) would contribute towards biomass production of vegetative parts. Thus, on the basis of the roles played by this nutrient and phytohormone, we could easily visualize their direct or indirect involvement in these structures. This, in turn, could be responsible for the improvement of plant height and root morphology; these improved lead the plants with better orientation of plants for harvesting the solar energy as well as for facilitating processes of plant metabolism. Thus, we may postulate that GA₃ supplemented with Ca⁺² in the present study was more effective in the enhancement of plant performance (Tables 1, 2; Figs 1, 2).

CONCLUDING REMARKS

The assessment of the results allows us to conclude that application of Ca⁺² alone as well as in combination with GA₃ increased plant growth and physiological attributes of faba bean. However, in Experiment 2, Ca⁺² supplemented with GA₃ was found more effective for the enhancement of growth attributes by in promoting photosynthetic pigments and CA activity than alone Ca⁺² application in Experiment 1.

REFERENCES

- Asahina M, Iwai H, Kikuchi A, Yamaguchi S, Kamiya Y, Kamada H, Satoh S (2002) Gibberellin produced in the cotyledon is required for cell division during tissue reunion in the cortex of cut cucumber and tomato hypocotyls. *Plant Physiology* **129**, 201-210
- Barnes JD, Balaguer L, Manrique E, Elvira S, Davison AW (1992) A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. *Environmental Experimental Botany* **32**, 85-100
- Bond DA, Lawes DA, Hawtin GC, Saxena MC, Stephens JS (1985) Faba bean (*Vicia faba* L.). In: Summerfield RJ, Roberts EH (Eds) *Grain Legume Crops*, William Collins Sons Co. Ltd., London, WIX 3LA, UK, pp 199-265
- Brian PW (2008) Effects of gibberellins on plant growth and development. *Biological Reviews* **34**, 37-77
- Broughton WJ, McComb AJ (1971) Changes in the pattern of enzyme development in gibberellin-treated pea internodes. *Annals of Botany* **35**, 213-228
- Bush DS (1995) Calcium regulation in plant cells and its role in signaling. *Annual Review of Plant Physiology and Molecular Biology* **46**, 95-122
- Dwivedi RS, Randhawa NS (1974) Evaluation of rapid test for hidden hunger of zinc in plants. *Plant and Soil* **40**, 45-451
- Easterwood GW (2002) Calcium's role in plant nutrition. *Fluid Journal Online Winter*, 1-3
- Evins WH, Varner JE (1972) Hormonal control of polyribosome formation in barley aleurone layers. *Plant Physiology* **49**, 348-352
- Fenn LB, Hasanein B, Burks CM (1995) Calcium-ammonium effects on growth and yield of small grains. *Agronomy Journal* **87**, 1041-1046
- Fleet CM, Sun TP (2005) A DELLAcate balance: the role of gibberellin in plant morphogenesis. *Current Opinion in Plant Biology* **8**, 77-85
- González L, González-Vilar M (2001) Determination of relative water content. In: Roger MJR (Ed) *Handbook of Plant Ecophysiology Techniques*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 207-212
- Hepler PK (2005) Calcium: a central regulator of plant growth and development. *The Plant Cell* **17**, 2142-2155
- Hirschi KD (2004) The calcium conundrum. Both versatile nutrient and specific signal. *Plant Physiology* **136**, 2438-2442
- Khan MN, Siddiqui MH, Mohammad F, Naem M, Khan MMA (2010) Calcium chloride and gibberellic acid protect linseed (*Linum usitatissimum* L.) from NaCl stress by inducing antioxidative defence system and osmoprotectant accumulation. *Acta Physiologia Plantarum* **32**, 121-132
- Lechowski Z, Białczyk J (1993) Calcium mediated cytokinin action on chlorophyll synthesis in isolated embryo of Scots pine. *Biologia Plantarum* **35**, 53-62
- Liang W, Wang M, Ai X (2009) The role of calcium in regulating photosynthesis and related physiological indexes of cucumber seedlings under low light intensity and suboptimal temperature stress. *Scientia Horticulturae* **123**, 34-38
- Marschner H (2002) *Mineral Nutrition of Higher Plants* (2nd Edn), Academic Press, London, total pp
- Mozer TJ (1980/77) Control of protein synthesis in barley aleurone layers by the plant hormones, gibberellic acid and abscisic acid. *The Cell* **20**, 479-485
- Nayek B, Biswas AK, Choudhuri MA (1983) Effect of calcium on water-stress-induced biochemical changes and yield of field-grown rice. *Biologia Plantarum* **25**, 117-123
- Pain SK, Dutta JK (1977) Studies on growth and metabolism of *Zea mays* L. I. The effect of application of gibberellic acid on the growth and metabolism of seedlings. *Indian Biology* **9**, 38-43
- Shabala S, Shabala L, Volkenburgh EV (2003) Effect of calcium on root development and root ion fluxes in salinised barley seedlings. *Functional Plant Biology* **30**, 507-514
- Siddiqui MH, Khan MN, Mohammad F, Khan MMA (2008) Role of nitrogen and gibberellin (GA₃) in the regulation of enzyme activities and in osmoprotectant accumulation in *Brassica juncea* L. under salt stress. *Journal of Agronomy and Crop Science* **194**, 214-224
- Silverstone AL, Sun T (2000) Gibberellins and the green revolution. *Trends in Plant Science* **5**, 1-2
- Slatyer RO (1967) *Plant-water relationships*, Academic Press, London, 366 pp
- Smith GS, Johnston CM, Cornforth IS (1983) Comparison of nutrient solutions for growth of plants in sand culture. *New Phytologist* **94**, 537-548
- Tamura S, Kuramochi H, Ishizawa K (2001) Involvement of calcium ion in the stimulated shoot elongation of arrowhead tubers under anaerobic conditions. *Plant and Cell Physiology* **42**, 717-722
- Tanimoto E (2005) Regulation of root growth by plant hormones – roles for auxin and gibberellin. *Critical Reviews in Plant Sciences* **24**, 249-265
- Vlitos AJ, Meudt W (1957) Relationship between shoot apex and effect of gibberellic acid on elongation of pea stems. *Nature* **180**, 284
- White PJ, Broadley MR (2003) Calcium in plants. *Annals of Botany* **92**, 487-511