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Relationship between Gibberellic Acid Addition and Protein, Yield and Symbiotic Nodulation in Pigeon Pea

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IN SHORT-SEASON pigeon pea production areas, low soil temperature is the major factor limiting pigeon pea establishmen, nodulation and nitrogen fixation. Gibberellic acid (GA) pretreatment of crop seeds and overcome low soil temperature inhibition of seed germination and seedling development. However, previous studies have found that the application of (GAs) decreased legume nodulation and nitrogen fixation under optimal growth conditions. A field experiment was conducted under short season conditions in the farm of Faculty of Agriculture in Assiut, Al-Azhar University, to determine whether the application of GA_3 to pigeon pea (*Cajanus cajan* L. Mill sp.) seed could accelerate germination and increase plant nodulation and nitrogen fixation. The results indicated that GA_3 application accelerated seedling emergence but decreased plant nodulation and nitrogen accumulation at early plant growth stages. However, these initial negative effects were overcome as the plants developed. Gibberellic acid applied to pigeon pea seed at the time of planting did not influence final grain and protein yield.

Keywords : Shart season, Protein, Yield, Nitrogen fixation, Grain yield, Gibberellic acid, Rhizobium, Pigeon pea (*Cajanus cajan* L. Mill sp.).

In the farm of Faculty of Agriculture in Assiut, Al-Azhar University, crop emergence can be delayed due to slow soil warming after planting. The seed-bed temperature at the customary planting time for most field crops is close to the biological minimum temperature for growth, approximately 10°, for many crops of tropical and subtropical origin. Low temperature reduces the emergence rate (Mock and McNeill 1979) and increase the susceptibility to seed and seedling diseases which reduce overall seedling vigour (Schulz and Bateman, 1968). Low temperature also reduces percent emergence (Dubetz *et al.*, 1962) resulting in poor stand establishment. At 25° (the optimal temperature for pigeon pea growth; Tyagi and Tripathi, 1983) pigeon pea seeds required 4 to 5 days to complete germination, and the germination rate reached at least 90%. At 15°, pigeon pea seeds required about 40 days to complete germination (maximum germination rate was 80%), while at 10° seeds germinated, very late and required nearly 15 days to complete the process, with the final germination percentage reduced to 60% of the 25° level.

Pigeon pea, in association with *Rhizobium leguminosarum* biovar viceae, can fix 100 to 200 kg N ha^{-1} yr^{-1} from the atmosphere (Smith and Hume, 1987). Since root zone temperatures, during the first month of the growing season in Assiut, pigeon pea production are often below 15°, all stages of pigeon pea nodulation and nodule function are negatively affected (Jones and Tisdale 1921, Lynch and Smith, 1994; Roughley and Date, 1986; Zhang and Smith, 1994). Barly nodule infection processes are the most sensitive to low temperature (Lynch and Smith 1993, Zhang and Smith, 1994). The plant to bacteria signal exchange, during early establishment of the pigeon pea *Rhizobium leguminosarum* biovar viceae symbiosis, is stronlgy inhibited by low root zone temperatures (RZTs) (Zhang and Smith, 1996).

Some studies have indicated that plant growth regulators (PGRs), such as gibberellic acids (GA_S), stimulate seed germination at low temperatures. Durrant and Mash (1991) reported that adding gibberllins (GA_{47}) to sugar-beet seeds (*Beta vulgaris* L. var. altissima) was beneficial to seed germination under cold, wet conditions. Recent studies at Al-Azhar University in Assiut indicated that GA_3 and kinetin were more beneficial to the germination of pigeon pea seeds at 10 than that at 1 5°C, while at 25°C; a temperature in the optimal range, neither GA_3 nor kinetin affected seed germination. The problem of low temperature and decreased germination of pigeon pea seeds under cool early season conditions could be, at least partly, addressed by appling GA_s or kinetin as a seed pretreatment.

However, under optimal pigeon pea growth conditions, daily foliar application of GA_3 delayed the formation of nodule initials and reduced the number, mass per nodule and specific activity of nodules by 43, 31 and 47%, respectively (Williams and Sicardi de Mallorca, 1984). Similar effects on nodulation resulted from the addition of GA_3 to the root medium (Williams and Sicardi de Mallorca, 1984). Evidence implicating the involvement of GA_3 in the regulation of nodulation is provided by the fact that this hormone is particularly concentrated in nodular tissue (Dullaart and Duba, 1970; Hardy *et al.*, 1973 and Williams & Sicardi de Mallorca, 1984) and its exogenous application to a number of lejumes has been shown to inhibit nodule formation (Fletcher *et al.*, 1958 and Williams &Sicardi de Mallorca, 1982 & 1984).

As described above, GA_S can increase seed germination under low soil temperature conditions, but have been shown to decrease pigeon pea nodulation and nodule function under more optimal growh conditions. The situation could be different at low soil temperatures. Zhang and Smith (1996) reported that low soil temperature reduced pigeon pea nodulation and nitroaen fixation by reducing the biosynthesis of the isoflavonoid genistein and decreasing plant root growth. Cibberellic acid application onto the corolla of *Petunia hybrida* increased anthocyanin synthesis and chalcone isomerase enzyme actvity (Weiss *et al.*. 1990). The increase in anthocyanin biosynthesis is correlated with the coordinated appearance of relevant enzymes such as phenylalanine ammonia-lyase (Weiss and Halevy,1989), chalcone flavanone isomerase (Weely *et al.*, 1.983). chalcone synthase (Rail and Hemleben, 1984) flavanone 3 hydroxylase (Dangelmayr *et al.*, 1983), and 3-O-flavonoid-glucosyltransferase (Cerats *et al.*, 1983). All of these enzymes ale also involved in the biosynthesis of isoflavonoids (Barz and Welle, 1992). Therefore, it is reasonable to suppose the GA application could increase pigeon pea nodulation and nitrogen fixation by increasing the activities of enzymes involved in the biosynthesis is isoflavoinoids and also by increasing seed germination and seedling development under low soil temperature conditions.

Earlier seedling emergence should also lead to earlier onset of photosynthesis which has the potential to overcome any possible negative effects of GA on nodulation. However, to date there have been no investigations of the effects of GA_S on pigeon pea nodulation and nodule function under short season field conditions. In this study, these hypotheses have been verified : 1) GA_3 applied to pigeon pea seeds at the time of planting increases pigeon pea nodulation and nodule function under Assiut short field conditions, 2) application of GA_3 increases pigeon pea grain and protein yield to improve plant nodulation and nitrogen fixation.

Material and Methods

Experimental site and design

The experiment was located at the farm of Faculty of Agriculture in Assiut, Al-Azhar University during the summer season of 2000. Two factors were included in this experiment: GA_3 level (equivalent to 0,90 and 900 mg/L;

based on a preliminary controlled environment experiment) and pigeon pea cultivar (P4785 and *Pusa* Ageti). The experiment was designed as a factorial randomized complete block with four replications. For each replication, one plot of a non-nodulating Evans line was included as control for estimating plant seasonal nitrogen fixation (N-difference method). Each plot (2x3m) consisted of four rows of plants with 40 cm between rows. The space between plots was 80 cm with 2 m between replications. In the previous year, 2000, this experimental field had been planted with wheat.

Gibberellic acid application and seed preparation

To estimate the amount of GA_3 to be applied to each of the seeds, one hundred pigeon pea seeds of each cultivar (from the same sources as the seeds used in field study) were weighed before and after complete inhibition. The amount of GA_3 to be applied to each of the seeds was the amount that would have been contained in that amount of water at 90 mg/L or 900 mg/L concentrations. This was calculated as follows: 90 (or 900) mg/L=90 (or 900) mg/900 mL = X /[(wet seeds- dry seeds)/ 100], Where X = the amount of GA_3 to be applied to each of the seeds for 90 mg/L (or 900 mg/L) treatment.

Anchor (Agricultural guarantee: carbathiin, 66.7g per L and thiram, 66.7g per L. Uniroyal Chemical. Elmira, Assiut) was used as a sticker for the GA_3. We found that we could add it in an amount equal to about 3% of the seed weight. Therefore, the correct amount of GA_3 was added to the correct amount of Anchor. and the resulting material were applied to the seeds at approximately 3% of the seed weights. Equal amounts were applied to the seeds of the control plots.

Seeds of the pigeon pea cultivars "P4785" and "Pusa Ageti" were selected in this study as they have been developed for production under the short season, cool conditions of Assiut and have performed well there. The seeds were planted by hand on July 20. In order to ensure that the plants were well nodulated, inoculum was produced by culturing *Rhizobium leguminosarum* biovar viceae strain ARC204 was obtained from Microbiology Research Center, Cairo Mircen, Egypt (EMCC), Fac. of Agric.. Ain Shams Univ., Cairo. Egypt, (Hume and Shelp, 1990) in yeast extract mannitol broth (Vincent, 1970). Twenty ml of inoculum per one meter of row were applied evenly by syringe directly onto the seed in the furrow. Following emergence seedlings were thinned to achieve stands of 500,000 plants/ha (20 plants/m of row, with an average inter-plant distance of 5 cm).

Data collection

Plant samples were harvested at four times, vegetative stage 3 (V3 (Fehr *et al.*, 1971), June 22), reproductive stage 1 (R1. June 29), reproductive stage 4 (R4, July 28) and reproductive 8 (R8. August 14) (Table 1), for investigation of leaf number, leaf area, plant height, nodule number and nodule weight. Leaf number and area per plant were determined using a Delta-Tarea meter (Delta-T Devices Ltd., Cambridge, UK). Plant shoots were oven-dried at 70°C for at least 48hr. and weighed. Plant shoot weight is expressed on a 0% moisture basis. The dried plant shoots from each plot were ground with a Moulinex coffee mill, for determination of nitrogen concentration. At the end of the growing season, grain yield was determined using plants from a two-meter row length taken from one of the two middle rows in each plot. Plants were harvested by hand at maturity and threshed with a plot combine (Wintersteiger, Salt Lake City, µT). oven-dried to a constant weight at 70°C, then weighed. Grain dry matter yield was also expressed on a 0% moisture basis. The dried seeds from each plot were ground using the same Moulinex coffee mill. The nitrogen concentrations of the shoots and grain were determined by Kjeldahl analysis (Kjeldtec system, Tecator AB, Hoganas, Sweden), (Page *et al.*,1982).

TABLE 1. Description of the four harvest stages.

Harvest time	Growth stage	Description
1	V3	Three nodes on the main stem with fully developed leaves beginning with the unifoliolate nodes.
2	R1	One open flower at any node on the main stem.
3	R4	Pods 2 cm long at one of the four uppermost nodes on the main stem with a fully developed leaf.
4	R8	Ninety-five percent of the pods have reached their mature pod colour.

Statistical analysis

Results were analyzed statistically by analysis of variance using the Statistical Analysis System (SAS) computer package (SAS Institute Inc., 1988; Snedecor & Cochran 1981 and Steel & Torrie, 1980).

Results

GA_3 effects on nodulation and nodule mass

Application of GA_3 to pigeon pea seeds affected pigeon pea nodulation at each of the four harvests (Fig. 1). At early plant growth stages, GA_3 application negatively affected nodulation. Both GA_3 treatments, 90 and 900 mg/L, decreased the number of nodules per plant for pusa Ageti at the first two harvested, and for P 4785 at the first harvest. The inhibition of pigeon pea nodulation by GA_3 diminished and finally disappeared as the plants developed. At the later growth stages, the lower GA_3 concentration (90 mg/L) had positive effects on nodule number and nodule dry weight per plant (Fig. 1 and 2).

At the first (onset of nitrogen fixation) and second harvests, nodule numbers of pusa Ageti plants were decreased by GA_3 application and the higher GA_3 concentration (900 mg/L) was more inhibitory (Fig. 1A). At the third harvest, the nodule number of Pusa Ageti plants receiving 90 mg/L GA_3 was no longer different from the 0 GA_3 control and by the final harvest, it was higher than the 0 GA_3 control. However, pusa Ageti plants receiving 900 mg/L GA_3 had a lower nodule number at the first three harvests, than the 0 mg/L GA_3 control plants, while at the last harvest there was no difference of nodule numbers between pusa Ageti plants receiving 900 mg/L and the 0 mg/L GA_3 control (Fig. 1A).

The effects of GA_3 on nodule number of P4785 plants followed the same general pattern as those of Pusa Ageti plants at the first harvest (Fig. 1B). The decrease in the nodule number of plants receiving 90 mg/L had recovered after the second harvest, with no differences between plants receiving 90 mg/L and 0 mg/L GA_3. As GA_3 concentration inereased, the time required for recovery of nodule numbers increased and nodule numbers were not different between plants receiving 90 mg/L and 0 mg/L GA_3 after the third harvest (Fig. 1 B).

There was an interaction between GA_3 application and pigeon pea cultivars. Gibberellic acid application decreased the total nodule mass of both Pusa Ageti and P4785 plants at the first two harvests (Fig. 2). For Pusa Ageti, these decreases in nodule mass continued through the third and founth harvests (Fig. 2A). However, at the last two harvests, the nodule weight per plant of P4785 receiving 90 mg/L GA_3 was higher when compared with plants receiving 0 GA_3.

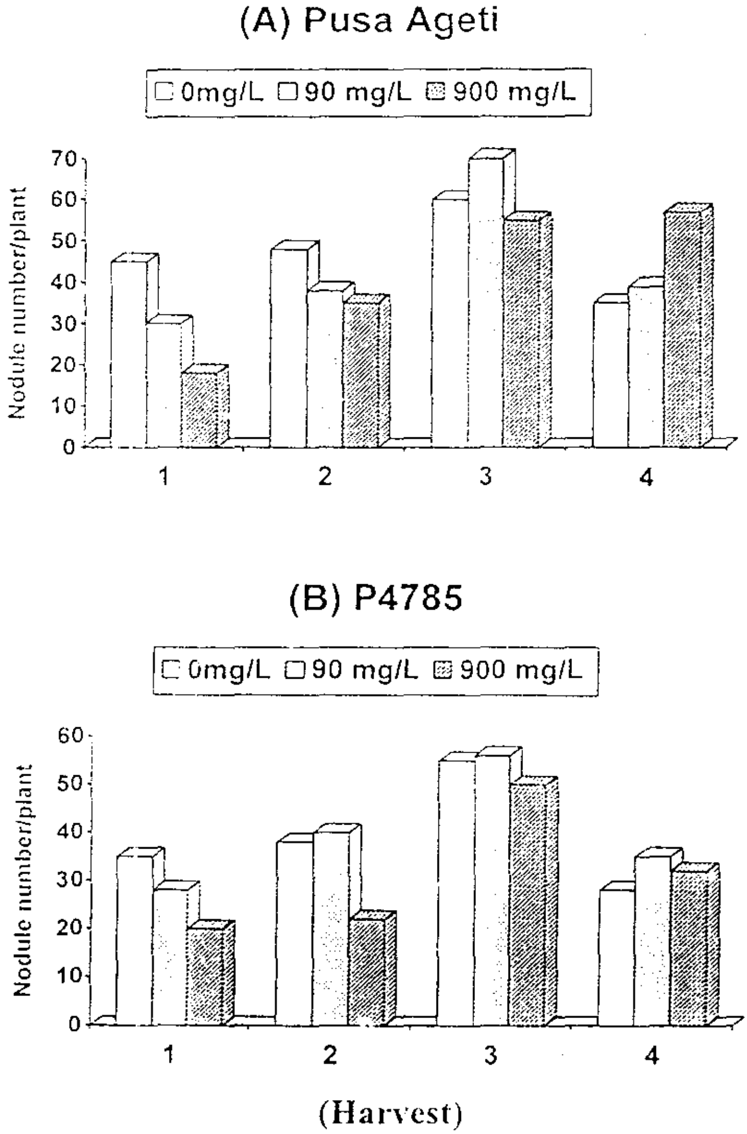

Fig. 1. The effects of GA_3 applied to pigeon pea seeds on nodule number per plant. Vertical lines on the top of each bar indicate one standard error Unit (n=8).

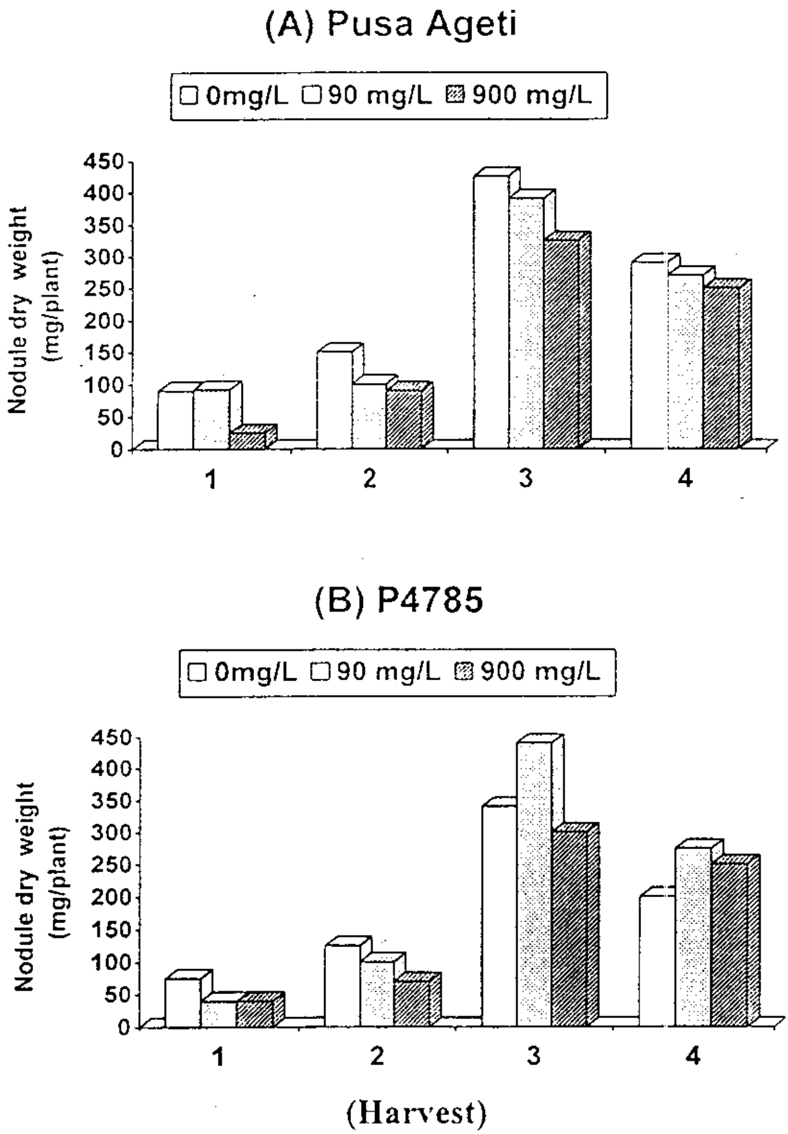

Fig. 2. The effects of GA_3 applied to pigeon pea seeds on plant nodule dry weight. Vertical lines on the top of each bar indicate one standard error Unit (n=8).

GA_3 effects on shoot nitrogen content

Gibberellic acic application decreased shoot nitrogen accumulation for pusa Ageti at the first three harvests, and for P4785 at the first two harvests (Fig. 3). As the plants developed, the negative effects of GA_3 application on plant shoot nitrogen content were also recovered. By the final harvest, the 900 mg/L GA_3 application resulted in increased shoot nitrogen content of Pusa Ageti (Fig. 3A). For P4785, 90 mg/L GA_3 increased shoot nitrogen content at the third harvest, with no differences among treatments at the final harvest (Fig. 3B).

Plant development

Gibberellic acid accelerated pigeon pea emergence (Table 2). The degree of acceleration was greater at 900 mg/L than 90 mg/L GA_3. The days between planting and 10%, 50%, and complete seedling emergence were shortened as much as 5,6 and 4 days , respectively (Table 2).

While it accelerated seedling development gibberellic acid application inhibited subsequent early plant growth. However, from the third havest on, 90 mg/L of GA_3 application increased the leaf areas of both P4785 and Pusa Ageti (Fig. 4A). For P4785, application of 900 mg/L GA_3 also increased leaf area as compared to the 0 gA_3 control at the last two havests, while this treatment increased leaf area only at last harvest for Pusa Ageti. Averaged over both pigeon pea cultivars, the green leaf number on September 9, of plants receiving GA at 900 and 90 mg/L were 10 and 5 per plant, respectively, while that of the non-GA treated plants was zero. The pod colour was also different between treatments. The pod colour of plants receiving 900 mg/L GA_3 and non GA application were light green, and brown, respectively, while the pod colour of plants receiving 90 mg/L GA_3 was between these two.

Although GA_3 application increased plant leaf area (Fig. 4) at later plant growth stages, and delayed plant senescence, plant shoot dry weights were not increased by GA_3 application.

Seasonal N_2 fixation grain and grain protein yield

Seasonal N_2 fixation by Pusa Ageti plants receiving the 900 mg/LGA_3 treatment decreased by 9.5%, while those of P4785 plants decreased by 5.3% (Fig. 3). The 90 mg/L GA_3 application did not affect the seasonal N_2 fixation of either Pusa Ageti or P4785 plants, and fixed nitrogen as percentage of total plant nitrogen content was not affected by either level of GA_3 application.

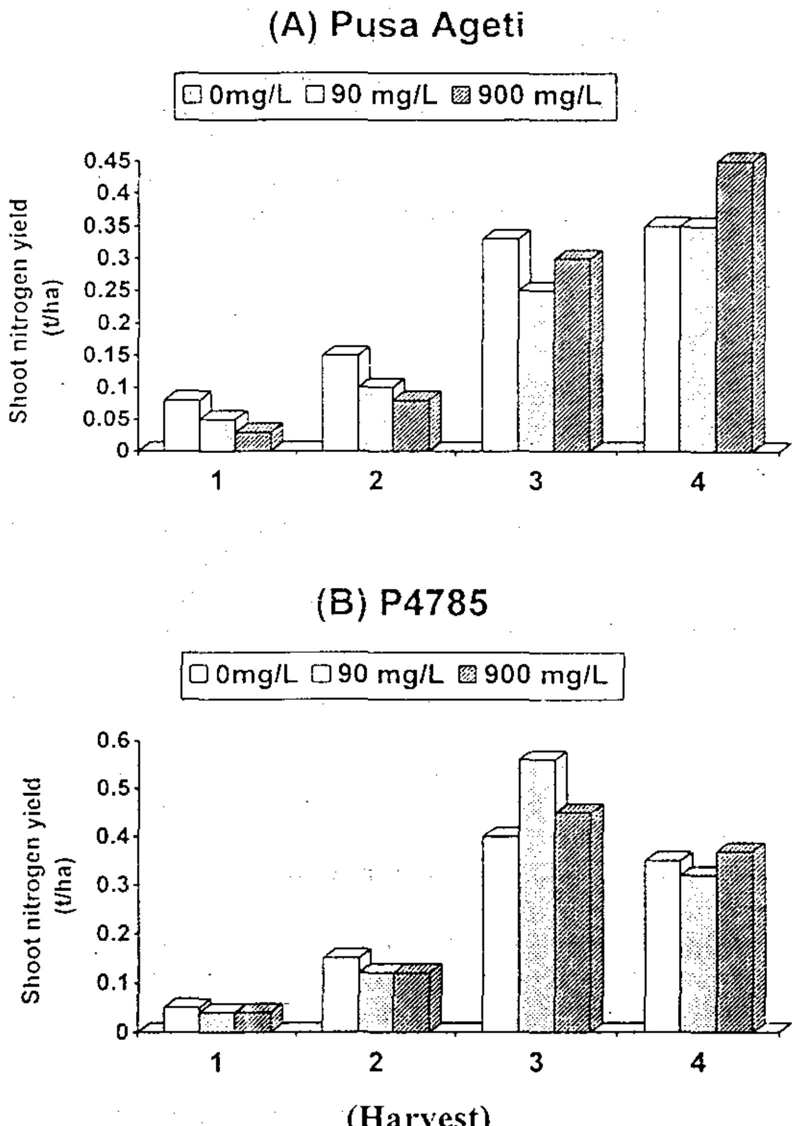

Fig. 3. The effects of GA_3 applied to pigeon pea seeds on shoot nitrogen per plant yield. Vertical lines on the top of each bar indicate one standard error Unit (n=8).

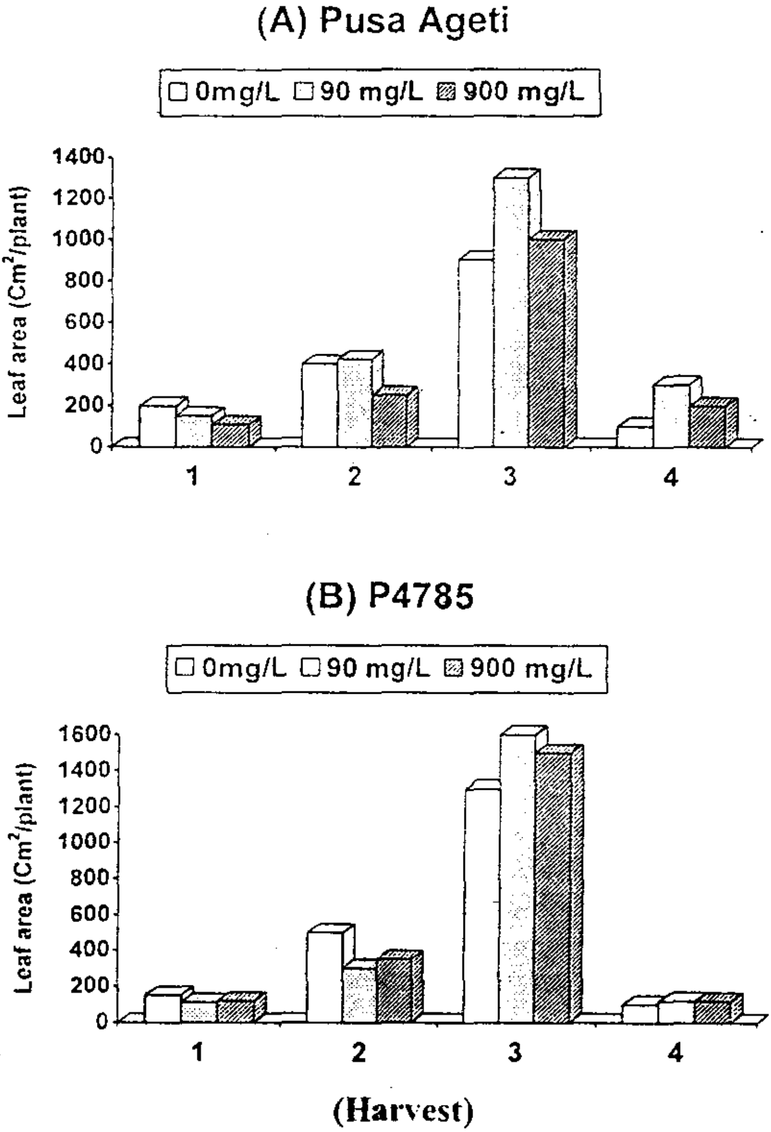

Fig. 4. The effects of GA_3 applied to pigeon pea seeds on leaf area per plant yield. Vertical lines on the top of each bar indicate one standard error Unit (n=8).

TABLE 2. Effects of gibberellic acid on pigeon pea in the short season area (date of planting was May 15, average of soil temperature was around 10°C).

Treatment	% Emergence		
	9% (days)	45%(days)	Full (days)
0 mg/L	12.4	14.7	16.0
90 mg/L	9.0	12.2	13.9
900 mg/L	7.4	9.8	12.0
L.S.D. at 0.05	0.7	1.1	2.0

Discussion

Although the exogenous application of GA_3 to pigeon pea seed accelerated the emergence of seedlings under low soil temperature conditions, it delayed subsequent plant development, and nodulation (Fig. 1), and decreased the nitsrogen fixation asbility of the plants (Fig. 3) during early plant development. Under field conditions, the emeraence of seeds treated with 90 mg/L GA was 1 to 2 days earlier than non-GA treated plants, with no statistically significant differences between 900 and 90 mg/L GA treated plants (Table 2). Application of GA_3 has previously been shown to reduce nodule numbers in *Phasoolus vulgaris* (Radley, 1961), *Vicia villosa* (Mes, 1959),*Trifolium hybridum, T. pratense, T. incarnatum and T. subterraneum* (Fletcher et al., 1958). The ability of GA_3 to reduce nodule numbers was decreased or eliminated by with holding its application until infection or nodule initiation had occurred but nodule size and effectiveness were still reduced (Williams and Sicardi de Mallorca, 1984). The reduction in nodule activity in GA_3 treated plants was not due to a directly inhibitory effect of the hormone on the nitrogenase enzyme (Singh *et al*.. 1981; Williams & Sicardi de Mallorca, 1984 and Abd-El-Ati *et al*., 2000). However, in our study, the decrease in nodulation and nitrogen fixation due to applying GA_3, to pigeon pea seeds were recovered as plants developed.

Gibberellic acid application inhibited plant growth and development at early growth stages, whereas it increased plant green leaf number and area at later growth stages (Fig. 4). A larae number of metabolic changes occur during leaf senescence, *e.g.* increases in the activity of protease (Thimann, 1980), glyoxysomal enzymes (Dashti *et al*., 1998 and Gut & Matile, 1988), and enzymes of chlorophyll catabolism (Hernandez and Focht, 1985 and Nock *et al*., 1992). As a consequence of this increase in enzymatic activities, the contents of protein, RNR and chlorophyll strongly decrease during plant senescence. Phytohormones like cytokinins, and in some cases gibberellin, delay the loss of chlorophyll whereas ethylene and abscisic acid enhance the rate of chlorophyll loss (Antoun *et al.*, 1998 and Thimann, 1980).

Although the application of GA_3 improved seed emergence (Table 2) and delayed plant senescence, plant dry matter accumulation (Fig. 4) and final grain and protein yield were not increased. These results may be due to the inhibition of early plant grwoth, nodulation, nitrogen fixation by GA_3 application (Fig. 1. 2, 3, and 4) .

The cultivar P4785 tended to be more responsive to GA_3 application (Fig. 1, 2, 3, and 4). P 4785 is a relatively eary-maturing cultivar in the farm of Faculty of Aariculture in Assiut, Al-Azhar University. Because application of GA_3 could increase enzyme activities, the contents of protein and RNA. and delayed chlorophyll senescence (Blank & McKeon. 1989; Malik *et al.*, 1997; Gulnaze *et al.*, 1999; ElSayed. 1998; Gut & Matile, 1988; Abdel-Mawly & El-Sayed. 1999; Ahlawat & Saraf, 1981; Nock *et al.*, 1992 and Thimann, 1980). after early pod development stages, GA_3 application resulted in higher per plant nodule number, shoot nitrogen content, and green leaf area of P4785 than Pusa Ageti.

Conclusion

In summary, this is the first report indicatina that when pigeon pea seeds are planted in cold soils, GA_3 pre-treatment of the seeds accelerates seedling emergence, but inhibits subsequent early growth and development. Nodulation and nitrogen fixation are decreased by GA_3 treatment during early plant development, but are increased during later development. The application of GA_3 increased plant nodulation and nitrogen accumulation after the early pod filling stage (R4). Gibberellie acid applied to pigeon pea seeds at the time of planting did not influence final grain and protein yield.

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العلاقة بين حمض الجبرليك المضاف والبروتين، والمحصول والعقد الحيوية فى بسلة الطيور

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أقيمت تجربة حقلية خلال الموسم الصيفى لعام ٢٠٠٠ بمزرعة كلية الزراعة- جامعة الازهر باسيوط لدراسة تأثير العلاقة بين منظم النمو (حمض الجبرليك) ودرجات الحرارة المنخفضة للتربية لانتاج بسلة الطيور وتكوينها للعقد البكتيرية والتثبيت الحيوى للنيتروجين وكذلك التغلب على الانخفاض فى درجات حرارة التربة والتى تمنع انبات البذور ونمو النباتات.

اظهرت النتائج انه حدث :

أولا : نقص فى عملية تكوين العقد البكتيرية وتراكم النيتروجين فى المراحل الأولى لنمو نباتات بسلة الطيور.

ثانيا : تلاشى هذا النقص فى عملية تكوين العقد البكتيرية تدريجيا ثم حدث زيادة فى تكوين العقد البكتيرية وزيادة فى عملية التثبيت الحيوى للنيتروجين.

كما أوضحت النتائج أنه نتيجة لإضافة حمض الجبرليك حدث زيادة فى إنبات البذور ونمو النباتات وتكوين العقد البكتيرية والتثبيت الحيوى للنتروجين، وكذلك أظهرت النتائج أن إضافة حمض الجبرليك لبذور بسلة الطيور فى نفس وقت الزراعة لا يؤثر على المحصول النهائى للحبوب وكذلك نسبة البروتين بها.