Morphology and Influence of Various Plant Growth Substances on Germination and Early Seedling Growth in *Macrotyloma uniflorum* (Lam.)

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Abstract

The paper presents the results of studies on morphological characters, seed germination and the influence of different concentrations of plant growth substances on *Macrotyloma uniflorum* including the comparative growth patterns of the seedlings. This is one of the lesser known beans mainly cultivated in hilly areas and commonly grown up at 1800msl. Seeds pre-soaked for 24h in various concentrations (0.1, 1.0 and 10 ppm) of GA_3, IBA and NAA respectively putting a separate control set soaked only in distilled water. The mean value of germination percentage, growth of root, shoot and cotyledonary expansion and biomass of seedlings were computed. The maximum germination percentage (99%) was observed through GA_3 0.1 and NAA 1 ppm in comparison to control set (90%). Although highest elongation of shoot was observed under GA_3 10 ppm (11.29 cm) and lowest under GA_3 at 1 ppm (10.45 cm) in comparison to control (7.50 cm) but the highest elongation of root was favoured by GA_3 at 0.1 ppm (4.46 cm) whereas, the minimum was observed under NAA at10 ppm (1.28 cm). The maximum Cotyledon expansion was gained in GA_3 at1ppm (5.36 cm) as compared to controlled set (2.94 cm). IBA 0.1 ppm resulted in highest dry weight of shoot (0.0183 g.) as compared to control (0.0172 g.) while dry weight of root was recorded highest under NAA at 10 ppm (0.0076 g). Lower concentrations of all the plant growth substances exhibited supportive response towards germination, growth of seedlings and development of cotyledons. The results of present findings show that applications of these plant growth substances if used at lower concentrations could enhance germinations and healthy seedling growth leading towards high production of *Macrotyloma uniflorum* to fulfill the demand of farming community. It is thus imperative that more identical studies be conducted on other legume species also generating data base so that the use of growth substances can be extended to produces healthy seedlings required to support large scale seed germination and plantations of those species. [Journal of American Science 2009;5(6):43-50]. (ISSN: 1545-1003).

Keyword: *Phaseolus mungo*, Morphology, Plant Growth Substances, Seed Germination, Seedling Growth.

Introduction

Production of high quality seeds is primary source to the success of Indian agriculture. Every farmer is sensitive to need for the rapid uniform seedling emergence and establishment of an even and productive stand. Crop production relies heavily on high quality planting seeds. The latest ISTA rules (ISTA, 2008) contain seed testing protocols of a large number of species cultivated all over the world and it forms the basic reference book for all kinds of seed testing activities and also for the international seed trade. Seeds are fundamental input to agriculture and natural ecosystem. The production of high percentage of viable seeds with capacity to germinate quickly is necessary for the propagation. Seed quality is very

essential for optimum stand establishment and maximum yield potential in soybean. As a result, it is necessary to have different seed testing parameters that permit rapid, objective and accurate evaluation of seed quality (S.C. Joshi *et al* 2009).

Horse gram *(Macrotyloma uniflorum* Lam) is a popular pulse, locally known as Gaheth belongs to the family Fabaceae that still remain an under exploited legume crop. It is usually grown up to the area at1800msl. Horse gram seeds are rich in protein and consumed in majority by poorest section of the society. The fodder being rich in protein; it is widely used as a feed to animals and horses (B. G. Prakash *et al.* 2008). Dehusking, germination, cooking, and roasting have been shown to produce beneficial effects on nutritional quality of both the legumes. Seeds and fruits of different species vary greatly in appearance, shape, size and ornamentation and structure of the embryo in relation to storage tissues. In some legumes seed coat colour is quite heterogeneous within a sample and such seeds often comprise a germplasm accession in a gene bank. Correlation of different seed coat colours with seed quality parameters may provide a non-destructive tool (especially useful in a gene bank) for sorting good quality seeds.

Germination represents a critical event in plant's life cycle and its timing largely predetermined the chances of survival of a seedling up to maturity (Thompson, 1973). Temperature is an important physical parameter of an environment, which determines the success or failure of a species in a particular locality, which in turns depends mostly on the germinability of the seed. It is well known that the different population of the same species varies in their temperature and light requirements for germination. Germination requirements of a particular species are a result of the interaction of its genetic makeup with the environment and dormancy pattern of seeds of various plant species, which enable them to survive during adverse conditions (Wittington, 1973; Nikolaeva, 1977). The germination of seed is affected by hormonal secretion and enzymatic activity with in the seeds. Gibberellins (GA) and Auxin- IBA (Indobutyric acid), IAA (Indole acetic acid) control many behavioral functions in plants which act as chemical messengers influencing many patterns of plant development. These stimulate cell division and cell elongation and control enzyme secretions. Seed germination and seedling growth can be influenced by various concentrations of growth regulators i.e. GA_3 and IAA (Chauhan *et al*. 2009).

The present investigation is carried out to investigate the response of different concentrations of the Plant Growth Substances on seed germination, root, shoot and hypocotyls elongation and biomass production of seedlings. Some morphological features of seed of Horse gram have also been observed with germination processes.

Material and Methods

The seeds of *Macrotyloma Uniflorum* were collected from Jakh village of District Tehri of Uttarakhad, India. The observation recorded on various morphological features of the seed such as shape, size, coloure, ornamentation, length and width, fresh and dry weight of seed, floral characters have been observed along with length and width of pod, pods per plant and seed per pod etc. After thorough mixing, the whole lot was sampled and dried in open air for ten days and stored at room temperature till the experimentation. The moisture content of seeds was determined by air oven method. Subsequently, the sample contained in glass and dried thermostatically at room temperature and weighed, then placed in hot air oven at 80^0 C for 48hrs to find out the average dry weight and calculated using the following formula (Anonymous, 1976).

$$\text{Moisture content (\%)} = \frac{\text{Fresh Weight - Dry weight}}{\text{Fresh weight}} \times 100$$

Seeds were pre-soaked for 24h under the different concentrations of Plant Growth Substances (0.1, 1.0 and 10 ppm) of GA_3, IBA and NAA respectively. A control set was soaked only in distilled water. The seeds were placed on a wet filter paper in petridish and kept in Seed Germinator. The mean valve of germination percentage, growth of root, shoot and cotyledonary expansion and biomass of seedlings were computed on each alternate day till the final day of experimentation (Table 1).

Table 1. The effects of various concentrations of different plant growth substances on seed germination and seedling growth of *Macrotyloma uniflorum*.

Plant Growth Substances	Concentrations used		
GA_3	0.1ppm	1ppm	10ppm
IBA	0.1ppm	1ppm	10ppm
NAA	0.1ppm	1ppm	10ppm
Control	Only distilled water		

Results and Discussion

Macrotyloma Uniflorum is an erect, sub-erect or trailing, densely hairy annual herb. The tap root produces a branched root system with smooth, rounded nodules. Seed is small somewhat gray to brown with pale fawn in colour sometimes with faint mottles or with small scattered black spots or with both. Seed size ranges 6-8 mm long and 3-4mm broad smooth of which 100 seed weight is recorded 3.65gm. The flowers are short and only 6-12 mm long cream - yellow with purple spot in auxiliary racemes with 2 appendages at base. The pods are 4-6 cm long and about 6 mm broad with 18-23 pods per plant bearing 6-7seeds per pod. Flowering and fruiting takes place between August to October (Table-2).

Correlation of different seed coat colours with seed quality parameters may provide a non-destructive tool (especially useful in a gene bank) for sorting good quality seeds. Relationship between seed coat colour and quality parameters in horse gram (*Macrotyloma uniflorum*) was studied by Singh et al. (2009) in seeds separated in to pale brown, medium brown and blackish brown coloured fractions in 20 accessions of horse gram. Seed quality was assessed in terms of germination, seedling vigour and seed storability. The light coloured fractions consistently showed highest germination percentage and seedling vigor followed by medium and dark coloured fractions in all the accessions. Percentage seed moisture content and electrical conductivity were observed to be highest in the dark coloured seeds. Light coloured seeds showed better storability after three years of ambient storage whereas the medium and dark coloured seeds were poor in germination and showed significant decline. Colour of seeds can be used as a visual indicator of seed quality and storability in horse gram.

Table 2. The Morphological Features of *Macrotyloma uniflorum* Seeds

S. No.	Parameters studied	Range of Variation
1.	Shape of seed	Seeds are small and somewhat round
2.	Colour	Gray to brown with pale fawn in colour. Sometimes with faint mottles or with small scattered black spots or with both.
3.	Ornamentation	Smooth and ovoid
4.	Length of seed	6-8 mm
5.	Width of seed	3-4 mm broad
6	Seed fresh weight (100)	3.65 gm
	Seed dried weight	1.095 gm
7	Flowers	Short only 6-12 mm long. The flower is cream - yellow with purple spot in auxiliary racemes with 2 appendages at base.
8	Pod	Shortly stipitate, slightly curved and tomentose.
9	Length and width of pod	4-6 cm long and about 6 mm broad with a point about 6 mm long.
10	Pods per plant	18-23 pods
11	Seed per pod	Usually bear 6 or 7 seed per pod.
12	The sowing time of the seed	Last week of June to first week of July.

	Flowering and fruiting period	The plant attains flowers and fruits between Aug to Oct.
13	Harvesting	The crop is harvested, when the seeds turn to shining brown-grey color, generally during mid Oct to Nov.

The observations of the experiment showed that GA_3-0.1ppm and NAA-1ppm resulted in equal 99 percent germination which was followed by IBA10ppm (96%) while control set showed lowest germination (90%). GA_3-1, GA3.10, IBA-0.1, and IBA-1ppm resulted in equal percent germination (98). Within the concentrations, the NAA-10ppm responded only 96 percent germination which is less than the control. (Table 3 and Fig. 1).

In case of the seedling growth, GA_3 at 10ppm attained maximum (11.29cm) shoot length which was followed by GA_3- at 1ppm (10.45cm) and IBA at 0.1ppm (9.23cm). Minimum shoot length was observed under NAA-10ppm (7.02cm) that was slightly lower than the control set (7.50cm). As far as root growth is concerned, it was observed highest under GA_3-at 0.1ppm (4.46) followed by NAA-at 1ppm (4.07). The lowest performance with regards to the root length was recorded under NAA-at 10ppm (1.28) that was very less than control (3.68).The cotyledon expansion was observed highest under the influence of GA_3-1ppm (5.36 cm) that was followed by GA_3-10ppm(3.79, Fig-4). The control set exhibited (2.94 cm) a little higher than NAA-10 ppm (2.27).

The highest cotyledons development has enhanced under the GA3 at 1ppm (5.36 ±0.5cm) concentration that was followed by GA3 at 100ppm(5.36 ±0.5cm) and GA3 0.1 ppm(3.65 ±0.3). The lowest growth was recoded under the influence of NAA-10ppm (2.27 ±0.2) which is less than the Control set (2.94 ±0.2, Fig-2). Dry weight of root biomass in case of controlled set was observed to be 0.0047±0.004g. The maximum dry weight of root was found in NAA at 10 PPM (0.0076 ±0.006g) and minimum dry weight of root was observed under GA_3 at 10 PPM (0.0021±0.002). The maximum fresh weight of shoot (0.0183 ±0.5) was observed under IBA at 0.1 ppm whereas, the dry weight of shoot under the control set has recorded higher (0.0172±0.2) than some of other concentrations (Table-4 and Fig.3).

Table 3: Effect of various concentrations of Plant Growth Substances on the seed germination and seedling development of *Macrotyloma uniflorum*.

Treatments	Germination %	Shoot Length (cm)	Root Length (cm)	Cotyledon Expansion (cm)
GA_3 0.1 ppm.	99	9.53 ±0.7	4.46 ±0.3	3.65 ±0.3
GA_3 1 ppm.	98	10.45 ±0.5	3.57 ±0.2	5.36 ±0.5
GA_3 10ppm.	98	11.29 ±0.8	3.69 ±0.5	3.79 ±0.2
IBA-0.1 ppm.	98	9.23 ±0.7	3.86 ±0.3	3.05 ±0.3
IBA -1 ppm.	98	8.34 ±0.7	3.95 ±0.3	2.91 ±0.2
IBA -10 ppm.	96	9.50 ±0.7	3.55 ±0.3	2.94 ±0.2
NAA- 0.1 ppm.	97	8.54 ±0.8	1.93 ±0.5	3.12 ±0.5
NAA -1 ppm	99	8.84 ±0.8	4.07 ±0.5	2.8 ±0.2
NAA -10ppm.	96	7.02 ±0.8	1.28 ±0.5	2.27 ±0.2
CONTROL	95	7.50 ±0.8	3.68 ±0.5	2.94 ±0.2

Table 4: Effect of various concentrations of Plant Growth Substances on the shoot and root dry weight of *Macrotyloma uniflorum*.

Treatments	Shoot Dry Weight(gm)	Root Dry Weight(gm)	Shoot: root ratio	Epicotyls length((cm)	No. of Roots
GA_3 0.1 ppm	0.0143 ±0.5	0.0053 ±0.005	1: 13	2.92	7
GA_3 1 ppm	0.0174 ±0.2	0.0029 ±0.002	1:0.8	2.62	9
GA_3 10ppm	0.0162 ±0.2	0.0021 ±0.002	1:0.2	3.52	9
IBA- 0.1 ppm	0.0183 ±0.5	0.0043 ±0.003	1: 4.1	3.46	8
IBA -1 ppm	0.0152 ±0.5	0.0036 ±0.003	1:3.6	3.44	8
IBA -10 ppm	0.0137 ±0.2	0.0053 ±0.005	1:3.5	1.87	8

NAA -0.1 ppm	0.0140±0.2	0.0045 ±0.004	1:0.6	2.45	7
NAA- 1 ppm	0.0143 ±0.2	0.0055 ±0. 005	1:40	2.58	7
NAA- 10 ppm	0.0175 ±0.5	0.0076 ±0. 006	1:1.8	0.29	6
Control	0.0172 ±0.2	0.0047 ±0. 004	1: 6.7	2.85	7

Heavy seeds generally have shown superior germination, survivorship, and seedling mass as also have been mentioned by Aaron M. Ellison (2001). The findings of Douglass H (1985) have summarized that seed size and germination requirements can be determined to be useful characters for resolving systematic and phylogenetic problems. Archna and Shivana (1985) have studied the requirements for seed germination and seedling formation of a hemi-root parasite *Sopubia delphinifolia*.

Fig. 1: Effect of Various Concentrations of Plant Growth Substances on the Seed Germination of *Macrotyloma uniflorum*

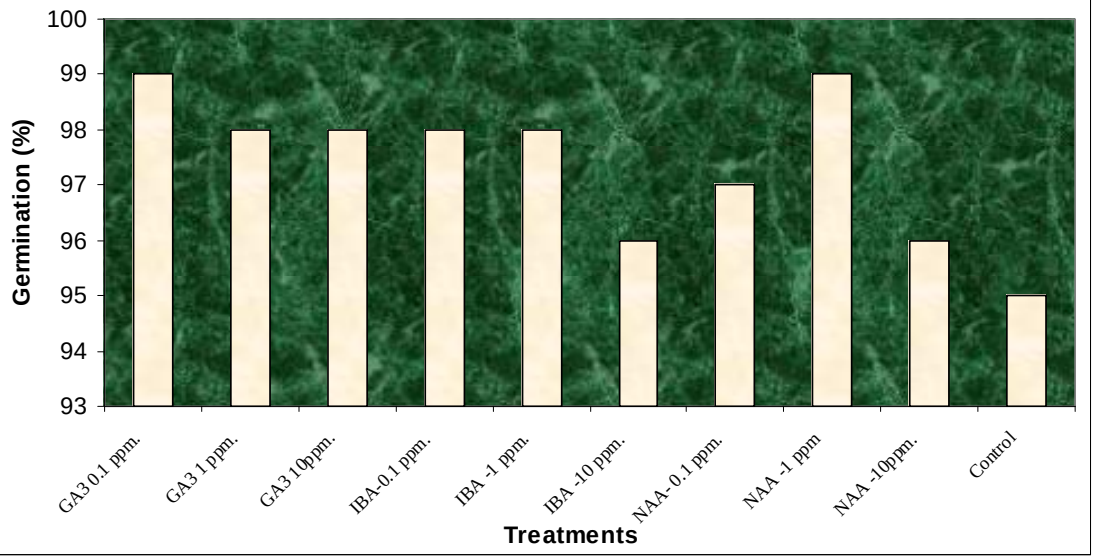
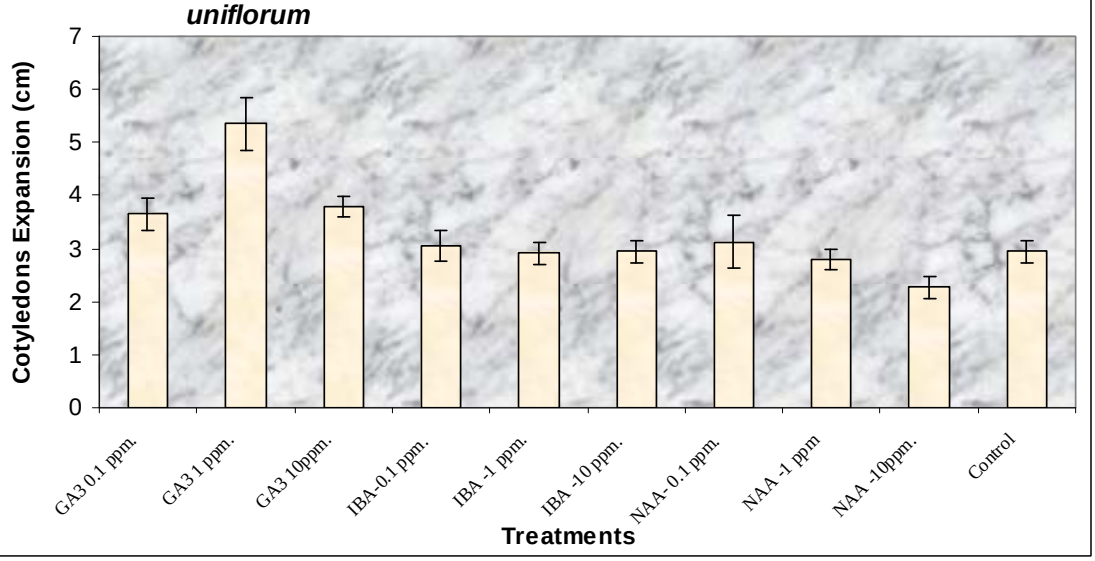


Fig. 2: Effect of Various Concentrations of Plant Growth Substances on the Cotyledons Expansion of *Macrotyloma uniflorum*



Light was found to be essential for germination; none of the growth substances could replace the light requirement. Seed must attain certain minimum specific moisture content, before they germinate (Negbi *et.al.*, 1966). GA_3 at 0.1ppm affected germination the most, leading 4% higher than the control. The more effective concentration has been proved to be GA_3 at 10 ppm with regards to shoot growth (11.29 ±0.8) at the cost of only 1% less germination. The same concentration resulted in almost equal growth of root (3.69 ±0.5) in comparison to control set. Response of lower and middle level of concentration such as GA_3 at 0.1ppm and NAA at 1ppm has shown remarkable germination and seedling growth in *Macrotyloma*. GA_3 resulted in maximum shoot growth (11.29±0.8) in comparison to Control (7.50±0.8). These concentrations have shown good response on cotyledons development (5.36±0.5). As far as dry weight of shoot and root is concerned, GA3 at10 ppm resulted less responsive in comparison to IBA 0.1 (0.0183±0.5) and $GA_3$1 ppm (0.0174±0.2).

Fig. 3: Effect of Various Concentrations of Plant Growth Substances on the Dry Weight of Shoots and Roots of *Macrotyloma uniflorum*

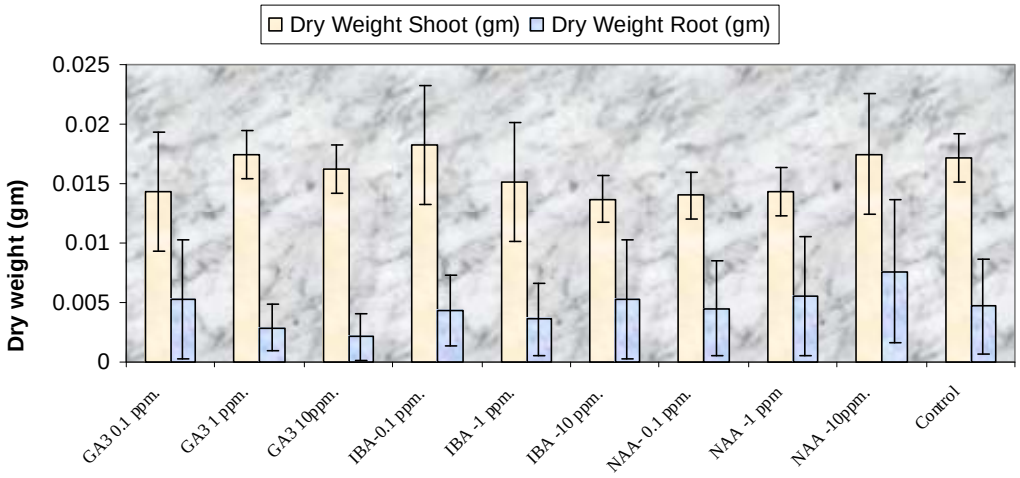
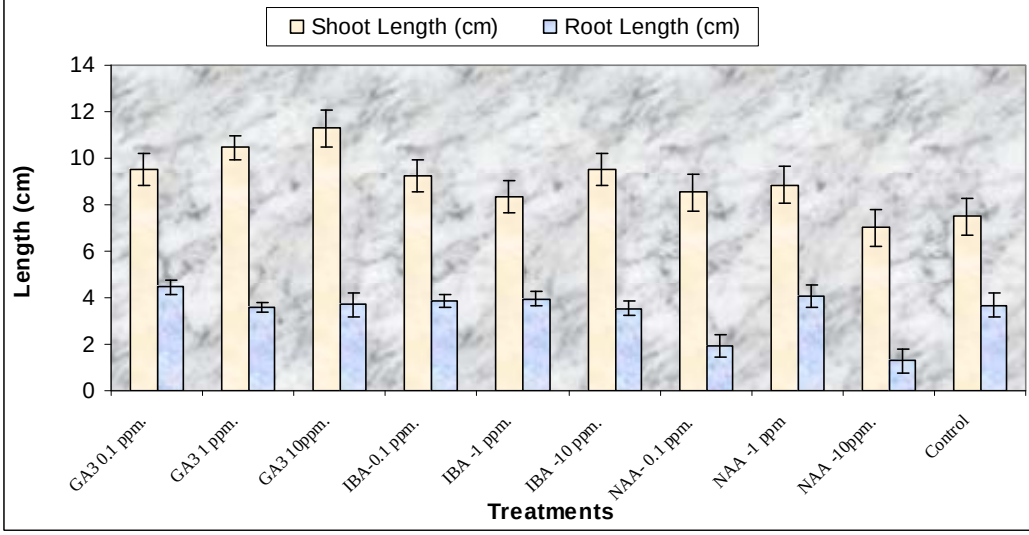


Fig. 4: Effect of Various Concentrations of Plant Growth Substances on the Length of Shoot and Root of *Macrotyloma uniflorum*



The effect of seed-soaking for 24 hours with different Plant Growth Substances has been examined by M. Grzesik. (2006) on the growth of seedlings of Lathyrus odoratus, Zinnia elegans, Matthiola incana and Antirrhinum majus. GA3 improved the germination of the treated seeds. The seedlings of Zinnia, Matthiola and Antirrhinum treated with NAA, GA3 and GA4+7 where higher, better branched and of better quality than the non-treated plants. Singh and Dara (1971) have shown the results on Influence of presoaking of seeds with gibberellins and Auxin on growth and yield attributes of wheat under high salinity, sodium adsorption ratio and boron levels. Similar efforts have been made through another experiment by M. Farooq et al. (2006) to investigate the possibility of rice seed invigoration by pre-sowing ethanol seed treatment. They revealed that employing ethanol treatments at lower concentrations can invigorate fine rice seeds. High temperature both delayed and inhibited the germinations of barley and radish seeds (Cavusoglu K & Kabar K 2007). Irfan Afzal et al., (2005) have shown the effects of seed soaking with plant growth regulators (IAA, GA3, and Kinetin) on wheat emergence and seedling growth under normal and saline conditions and found their usefulness in increasing relative salt-tolerance. Among the 14 pre-sowing treatments, KNO3 (150 min) and NaHClO3 (30 min) significantly stimulated seed germination of Angelica glauca and reduced mean germination time under both laboratory and nursery trials, as well as developed seedling vigour under nursery conditions (Jitendra S. Butola and Hemant K. Badola 2004). A similar experiment was carried out by Gao Huan Zhang et al. (2002) in which Walnuts cv. Jianshi were soaked in water (control), IBA at 80 mg/kg, IAA at 100 mg/kg, NAA at 80 mg/kg, ABT root-growing powder at 1 g/kg, or 6-BA at 5 mg/kg. In this experiment, different concentrations of GA3, IBA and NAA, the maximum germination percentage (100%) was recorded in seed treated with IBA 0.1 ppm, which shows that the lower concentrations of growth hormone shows better performance than higher which was similar to the results of James Chukwuma Ogbonna and P.G. Abraham (1989).

The results of the present findings are parallel to the findings of Parameswari and Srimathi (2008) to evaluate the influence of plant growth substances on seed germination and seedling quality characteristics of tamarind seeds. They revealed that the concentration of 100 and 200 ppm GA3 were found to enhance both the seed germination and seedling vigour parameters, such as root length, shoot length, hypocotyl length, dry matter production and vigour index values. Seed fortification with GA3 100 ppm performed well even under nursery conditions recording a higher leaf number and stem circumference.

IBA proved good in seedlings biomass production. However, lower concentrations of all the plant growth substances exhibited supportive response towards germination, growth of seedlings and development of cotyledons. The results of present findings shows that applications of these plant growth substances if used at lower concentrations could enhance germinations and healthy seedling growth leading towards the production of Macrotyloma uniflorum to fulfill the demand of farming community. It is thus imperative that more identical studies be conducted on other legume species also generating data base so that the use of growth substances can be extended to produces healthy seedlings required to support large scale seed germination and plantations of those species.

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