

EFFECTS OF SEED TREATMENT WITH GROWTH HORMONES AND RHIZOBIUM ON THE OIL CONTENTS, NITROGEN FIXATION AND YIELD OF SOYBEAN

ZARRIN FATIMA AND ASGHARI BANO

*Department of Biological Sciences,
Quaid-e-Azam University, Islamabad, Pakistan.*

Abstract

Effects of soaking seed with plant growth regulators viz., Indole acetic acid (IAA), Abscisic acid (ABA) and Kinetin each at 10^{-6} M on *Glycine max* (L.) cv. NARC-1 was studied. Kinetin was found to be most effective in increasing the yield, oil as well as ash contents. Greater seed oil content were found in ABA and Kinetin treatment. Maximum ash contents and seed weight were found in Kinetin treated seed as compared to control. The absorbance for ethylene at 412 nm of nodules was found to be significantly different from that of the control with maximum value recorded in Kinetin and minimum in IAA treated seeds.

Introduction

Soybean is considered as one of the oldest food crops of the world due to its good quality oil, protein contents and soil enriching properties. Oil and fats are essential items in the human diet, besides providing energy they improve the taste and platability of food. Oils and fat also play an integral role in cell formation and the function of cell membrane (Gandhi *et al.*, 1985). There are reports where seed treatment with ABA increased grain yield in water stressed plants of sorghum (Troare & Sullivan, 1990). Experiments were carried out to study the effects of soaking of soybean seeds with growth regulators on oil content, nitrogen fixation and yield.

Materials and Methods

Seeds of soybean (*Glycine max* L.) cv. NARC-1 were surface sterilized with 0.1% Mercuric chloride solution for 2 min. Seeds were then soaked in aqueous solution of IAA, ABA and kinetin (each at 10^{-6} M) for 6 h and then inoculated with *Rhizobium japonicum* strain where 1 kg seeds were inoculated by wetting with 16g of inoculum in 48% sugar solution in large Petri plate and thoroughly mixed to provide a uniform coating of inoculum on the seeds. Seeds soaked in distilled water served as control. The seeds were sown in earthen pots measuring 24x30 cm² filled with mixture of sand and soil in 1:3 ratio and kept under natural environment during mid August till maturity. During this period the photoperiod was 16 h and the temperature ranged from 27-38°C night/day and relative humidity varied from 61-81%. Organic manure and DAP (Diammonium phosphate) 1:10, in each pot were also added.

Nuclear magnetic resonance (NMR) was used to analyze the oil content of seeds of treatment and control plants at harvest following the method of Baily (1977) and Robertson & Windkam (1981). Ash content of seeds from treated and control plants was

determined at harvest following the procedure of AOAC (Anon., 1982). Ten week old plants were harvested for nitrogenase activity using absorption for C_2H_4 at 412 nm at flowering stage.

Nodules 1g were incubated in 30 ml McCartney's vials with rubber stopper, 2 ml of air was removed from the vial with syringe and 2 ml of acetylene were injected. Plant nodules were incubated with acetylene for 90 min., at 22°C, thereafter, 2 ml of gas phase were removed and injected in McCartney's vials containing 1.5 ml of oxidant solution (40 ml of 0.05 M $NaIO_4$, 5 ml of 0.001 M $KMnO_4$, adjusted to pH 7.5 with KOH, diluted to 100 ml). The absorbance for C_2H_4 at 412 nm was determined after 60 min. A blank was prepared with all the reagents, incubated and mixed for the same period and absorbance value of the sample was subtracted from that of blank (Larue & Kurz, 1973).

Results and Discussion

Some of the plant physico-chemical parameters as affected by growth regulators and *Rhizobium* inoculation on soybean are presented in Table 1.

Seeds weight: The DMRT showed that the seed weight plant⁻¹ significantly differed ($P < 0.05$) among the treatments as compared to control. However, IAA and ABA were non-significantly different from the control. Kinetin showed 36.4% maximum yield and large sized seeds than that of control. The minimum value was recorded in ABA (16.76%) and IAA (13.9%) treated seeds as compared to control. Maximum seed weight and large seed size in kinetin treatment may be attributed to increased translocation of assimilates from vegetative to reproductive stage. ABA inhibits α amylase synthesis by inhibiting the accumulation of translatable mRNA rather than the translation of existing mRNA as has been suggested by HO & Varner (1976).

Oil Content of Seeds: Data of oil content of seeds when subjected to ANOVA showed significant differences ($P < 0.01$) amongst the treatments. A significant increase ($P < 0.05$) in oil content of seeds was due to kinetin 3.62% and ABA 2.95% treatments as compared to IAA 2%. This is in accordance with the report of Ruth & Chris (1989) which showed that embryo lipid content increased under high exogenous ABA with preferential accumulation of eicosenoic and erucic acids, the predominant fatty acid constituents.

Ash content of seeds: DMRT revealed significant differences ($P < 0.05$) due to all three growth hormones than that of control. Maximum increase (2.25%) in ash content was found in kinetin treatment. This may be due to growth promoting effect of kinetin-induced increase in dry matter production. According to Berridge & Ralph (1971) kinetin mobilized the starch reserves and increased the flow of sugar in Chinese cabbage. The minimum ash content was found in IAA (-11.26%) and ABA (-10.6%) treated seeds.

Nitrogenase Activity (Absorbance for C_2H_4 at 412 nm): Maximum value was recorded in kinetin treated seeds (17.85%) as compared to ABA (10.7%) and IAA (-64.2%). The IAA-treated seeds showed minimum value. The value for kinetin and ABA-treated seeds was significantly lower than that of control and IAA effect was significantly higher than that of the control.

Table 1. Plant Physico-chemical parameters as affected by growth regulators and *Rhizobium* inoculation on Soybean.

Treatments	Nitrogenase activity of nodules (O-D at 412 nm)	Oil content of seeds plant ⁻¹ (g)	Seed weight plant ⁻¹ (g)	Ash content of seeds plant ⁻¹ (g)
Kinetin	0.3300a	18.59a	0.5483a	4.310a
ABA	0.2500c	18.47a	0.3343b	3.765b
IAA	0.1000d	18.30b	0.3456b	3.740b
Control	0.2800b	17.94c	0.4017b	4.215a

All such means which share a common letter are non-significantly different, otherwise they differ significantly at $P < 0.05$ in accordance with DMRT.

Musgrave *et al.*, (1982) and Raskin & Kenda (1984) have reported that GA and Kinetin cooperate with ethylene alleviating many of the stresses in rice and other plants at various developmental stages. Bano & Hillman (1986) reported that the development of root nodules was variously effected by ABA treatment, type of magnitude of the effect depend on the developmental phases of the root nodules at the time of ABA treatment.

References

- Anonymous. 1982. *Official methods for the determination of nitrogen and protein*. 11th edition, Association of official Analytical Chemists, Washington, DC pp. 788-789.
- Baily, R.F. 1977. The use of nuclear magnetic resonance as a replacement of solvent extraction techniques. *Appl. Spectrum. Madde Resonance Magn. Nucl. Ind. Abment*, 15: 289-298.
- Bano, A. and J.R. Hillman. 1986. Effect of Abscisic acid on nodule morphology, nitrogenase activity and hydrogen evolution in *Faba vulgaris*. *Annals of Botany*, 58: 281-283.
- Berridge, M.V. and R.K. Ralph. 1971. Kinetin and carbohydrates metabolism in Chinese cabbage. *Plant Physiol.*, 47: 562-567.
- Dure, L.S. 1975. Seed Formation. *Ann. Rev. Plant Physiol.*, 26: 259-78.
- Gandhi, A.P., M.M. Nenwant and A. Nawab. 1985. Some physio-chemical characteristics of soybean. *Food Chemistry*, 17: 71-74.
- HO, D.T. and J.E. Varner. 1976. Response of barley aleurone layers to abscisic acid. *Plant Physiol.*, 57: 175-78.
- Larue, T.A and W.G. Kurz. 1973. Estimation of nitrogenase using a calorimetric determination for ethylene. *Plant Physiol.*, 51: 1074-1075.
- Musgrave, A., Jackson and E.Ling. 1982. Callitriche stem elongation is controlled by ethylene and gibberellin. *Biol. Rev.*, 31: 109.
- Raskin, I. and H. Kenda. 1984. Role of gibberellin in the growth response of submerged deep water rice. *Plant physiol.*, 76: 947-950.
- Robertson, J.A and W.R. Windkam. 1981. A comparative study of methods of determining oil Content of Sunflower seed. *J. Am. Chem. Soc.*, 58: 993-996.

- Ruth, F. and S. Chris. 1989. Absciscic acid or high osmoticum promote accumulation of long chain fatty acid in developing embryo of *Brassica napus*. *Plant Science*, 61: 213-217.
- Troare, M. and C. Sullivan. 1990. Effect of Absciscic acid and seed treatment on sorghum drought responses. *Plant Physiol & Biochem.*, 2: 849-853.

(Received for publication 12 August, 1996)