

UPTAKE AND DISTRIBUTION PATTERN OF APPLIED ABSCISIC ACID (^3H -ABA) IN NORMAL AND HYPERNODULATING SOYBEAN VARIETIES

Muhammad Asim, Muhammad Asif, Muhammad Munir* and Muhammad Aslam**

ABSTRACT: The uptake and distribution of abscisic acid (ABA) were studied in inoculated and uninoculated plants of soybean [*Glycine max* (L.) Merr.] from the time of inoculation to root-nodule initiation (192 hours after inoculation). ^3H -ABA was applied to the leaves of two soybean cultivars: cv. Williams-82 and its hypernodulating mutant, NODI-3. There was a significant difference in the percent uptake of ^3H -ABA between the two varieties both in inoculated and uninoculated plants. A marked difference in the percent distribution of ^3H -ABA between different parts of the plants was observed at different developmental phases of root nodules. Leaves of NODI-3 retained more radioactivity as compared to Williams-82 in inoculated as well as uninoculated plants. The stem and roots of Williams-82 plants had a percent distribution of ABA that was higher than NODI-3. The phloem sap of Williams-82 had higher accumulation of ^3H -ABA at all stages after treatment. The basal levels of ABA in phloem sap were lower in NODI-3 than in Williams-82. Inoculation further accentuated this difference. The major changes in ABA level in phloem sap and leaves occurred between 48 to 96 h after inoculation.

Key Words: Soybean; Varieties; Absciscic Acid; Inoculation; Uptake; Distribution; Pakistan.

INTRODUCTION

Plant hormones are essential in almost every aspect of plant development and organogenesis; thus it is reasonable to speculate that they are also involved in nodule development at different growth stages of plant development (Ding and Oldroyd, 2009). Four plant hormones – auxin, cytokinin, gibberellin and abscisic acid – are found at concentrations that are higher in nodules than in uninoculated roots (Hirsch and Fang, 1994). Uptake and the characteristics of distribution of radiolabelled plant hormones have been studied in nodulated plants (Delrot et al., 1981, Suzuki et al., 2004) during different developmental phases of nodules including the morphogenetic phase (Bano and Hillman, 1989, Cooper and Long, 1994). It has long been postulated (Delves et al., 1986, Sheng and Harper, 1997) that the development of soybean–Rhizobium symbiosis is under the control of shoot and root factors. Several studies (Hirsch, 1992; Hirsch et al., 1997., Oldroyd and Downie, 2008) led to the hy-

pothesis that changes in the phytohormone balance are a necessary requirement to elicit nodule formation. Studies have documented high levels of ABA in the nodules as compared to roots (Watts et al., 1983) however, information is lacking on the uptake and distribution patterns of applied plant growth regulators during the phase of nodule initiation. Based on this, the study was formulated to quantify the uptake of applied radiolabelled abscisic acid (^3H -ABA) and to understand the distribution pattern of this applied ^3H -ABA in phloem sap, root, stem, and leaves of two soybean lines (normal and hypernodulated) in response to inoculation. The study was also aimed at investigating the distribution of ^3H -ABA during the phase of nodule initiation.

MATERIALS AND METHODS

Bacterial Strain and Culture

Bradyrhizobium japonicum strain USDA 110 was grown in yeast mannitol broth for

*Pakistan Agricultural Research Council, Islamabad, Pakistan.

** Ministry of Food and Agriculture, Islamabad, Pakistan.

5 d at 28 °C and 90 rpm on a shaker. Pots (six plants /pot) were inoculated with 10 ml of inoculum. Six pots remained uninoculated for control. The plants were harvested at various time intervals (0 h, 48 h, 72 h and 96 h after inoculation) until the appearance of visible nodules (192 h after inoculation).

Application of ^3H -ABA

For the study of uptake and transport of ^3H -ABA, 10 μl of ^3H -ABA (=800 cpm / μl) was applied to the leaves with micro syringe between 1000 and 1200h. The ^3H -ABA was applied 2h after inoculation in the inoculated treatments.

Collection of Phloem Sap

Shoots of six plants were severed 3 cm below the cotyledonary node using a sharp razor blade against a paper towel bath in a solution of 10 mM disodium EDTA with 5 mM phosphate buffer (pH 6.0). Plant shoots were transferred to micro centrifuge tubes containing 1ml of 100 mM disodium EDTA (pH 7.0). The micro centrifuge tubes holding the shoots were placed in a water bath (25 °C) lined with H_2O saturated paper towels and then covered to maintain high humidity and darkness for 2 h EDTA pretreatment. After 2 h, plant shoots were removed from the pretreatment solution and thoroughly rinsed in Reverse Osmosis (RO) purified H_2O . Plant shoots were transferred to 1.5 ml micro centrifuge tubes containing 1 ml of RO purified H_2O and returned to the water bath for phloem collection. After 4 h, the collection period was terminated by removing and discarding the plant shoot. The solutions containing phloem exudates were pooled and stored at -20°C until further analyses.

Extraction from Leaves, Stem and Root

The plants in both the inoculated and uninoculated treatments were harvested at 0 h, 48 h, 72 h, 96 h and 192 h after application of the labeled abscisic acid. The plants were divided into leaf, stem, and root portions. Each plant part was homogenized

separately with a mortar and pestle using butylated hydroxy toluene (BHT) as an antioxidant in 80% methanol. The extraction of each plant part was made for 72 h with concomitant change in the solvent after every 24 h. Thereafter, 1 ml of the supernatant was taken in a scintillation vial and 4 ml of scintillate {(toluene: PPO) [poly (2,6-dimethyl- 1,4 phenylene oxide) was added to it prior to counting in a scintillation counter.

Uptake and Transport of ^3H -ABA

A blank was prepared containing 1-2 μl ^3H -ABA. The counts in the sample were corrected against the blank. Percentage of ^3H -ABA was calculated following the equation:

$$\text{Percentage of } ^3\text{H-ABA taken by the whole plant} = \frac{\text{Total amount of } ^3\text{H-ABA taken in different parts/}}{\text{Total amount of } ^3\text{H-ABA applied}}$$

The percentage distribution of ^3H -ABA in different parts of a plant is determined according to the equation:

$$\text{Percentage of } ^3\text{H-ABA transported in particular part (stem, root, leaves)} = \frac{\text{Amount of } ^3\text{H-ABA calculated in that part of plant/}}{\text{Total amount of } ^3\text{H-ABA calculated in different parts of the plant in that sample}}$$

RESULTS AND DISCUSSION

Leaf ABA

The ABA level in leaves was similar in both the cultivars at the time of inoculation (0h), but decreased markedly in Williams-82 following inoculation and remained low in Williams-82 at all phases after inoculation (Figure 1 a, b) until the time of nodule initiation (192 h) when leaves of NODI-3 retained less amount of ^3H -ABA as compared to normal nodulating cultivar. In contrast, the ABA level in NODI-3 decreased through 48h after inoculation but remained high through nodule developmental phases (Figure 1a, b). There was

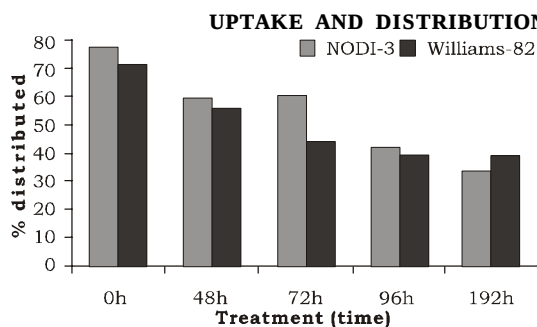


Figure 1a. Percentage distribution of ^3H -ABA in the leaves of inoculated plants of Williams-82 and its hypernodulating mutant, NODI-3

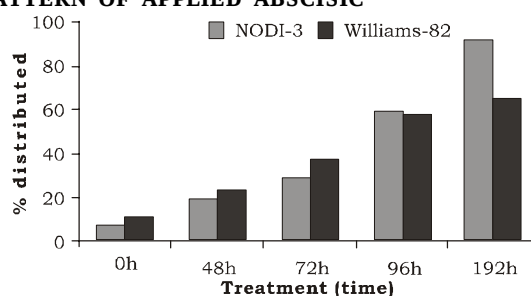


Figure 2a. Percentage distribution of ^3H -ABA in phloem sap of inoculated plants of Williams-82 and its hypernodulating mutant, NODI-3

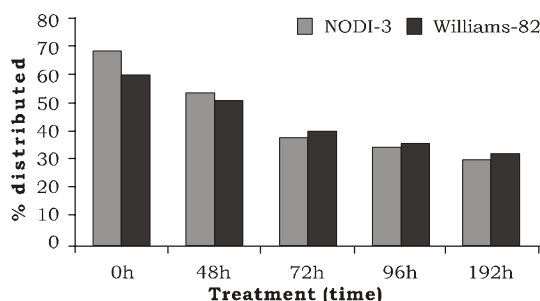


Figure 1b. Percentage distribution of ^3H -ABA in the leaves of uninoculated plants of Williams-82 and its hypernodulating mutant, NODI-3

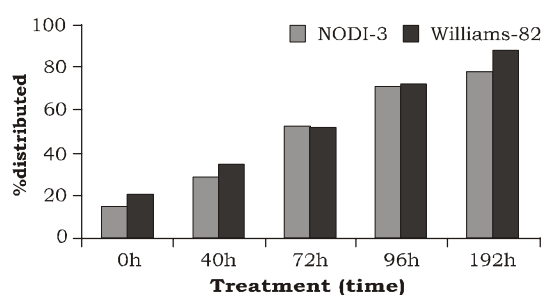


Figure 2b. Percentage distribution of ^3H -ABA in phloem sap of uninoculated plants of Williams-82 and its hypernodulating mutant, NODI-3

no marked difference in the ABA concentration in Williams-82 from 72h to 192h. The ABA level in NODI-3 was markedly higher at 48h and 72h after inoculation. ABA decreased markedly at nodule initiation, which was 192h after inoculation.

Phloem ABA

There were marked changes in ABA concentration of control (uninoculated) plants of Williams-82 through 96 h, following which the ABA concentration remained the same till 192h. The ABA concentration of NODI-3 control plants increased continuously to nodule initiation (192 h after inoculation); at that time it was almost double as compared to Williams-82. Nevertheless, the ABA concentration of uninoculated plants of NODI-3 was relatively same or less than that of the Williams-82 parent (Figure 2a, b).

Stem ABA

In response to inoculation, the ABA concentration in Williams-82 remained high through 72 h, then decreased and remained low through nodule initiation (192 h after inoculation) (Figure 3a). In contrast, ABA concentration increased 48h after inoculation, then decreased at 72 h and from there showed an inclining trend and remained high until the appearance of nodules at 192 h after inoculation, where a decrease in the ABA occurred in Williams-82 in response to inoculation. The ABA concentration of two cultivars was similar in the uninoculated ones, where 72h after inoculation it increased and then declined through to nodule initiation (192 h) (Figure 3a, b).

Root ABA

The ABA was higher in Williams-82 than in NODI-3 at 0 h before inoculation

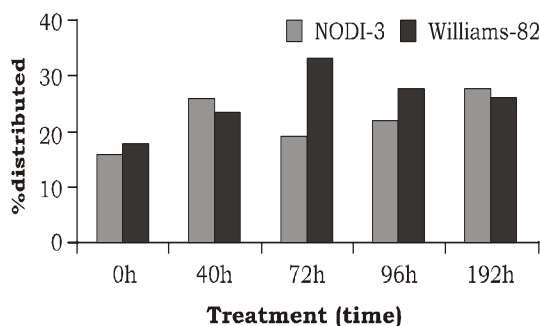


Figure 3a. Percentage distribution of ^3H -ABA in stem of inoculated plants of Williams-82 and its hypernodulating mutant, NODI-3

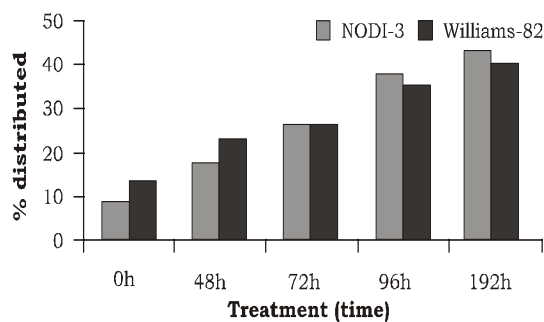


Figure 4a. Percentage distribution of ^3H -ABA in roots of inoculated plants of Williams-82 and its hypernodulating mutant, NODI-3

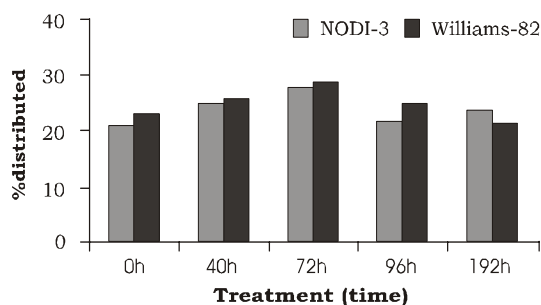


Figure 3b. Percentage distribution of ^3H -ABA in stem of uninoculated plants of Williams-82 and its hypernodulating mutant, NODI-3

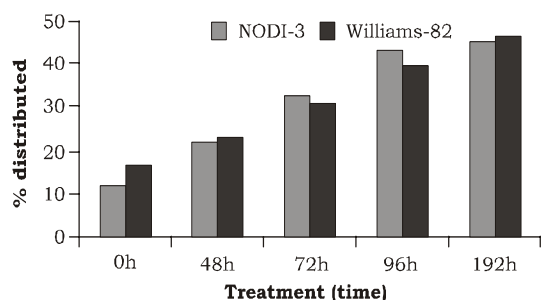


Figure 4b. Percentage distribution of ^3H -ABA in roots of uninoculated plants of Williams-82 and its hypernodulating mutant, NODI-3

(Figure 4a, b). In response to inoculation the ABA increased in NODI-3 to a level similar to that of Williams-82, at 72 h after inoculation and remained at higher levels at subsequent sampling times. At nodule initiation (192 h after inoculation) ABA in NODI-3 was greater than that of Williams-82. In contrast, ABA levels of uninoculated plants of NODI-3 at 72 h after inoculation become higher than that of Williams-82. This showed that NODI-3 retained a higher ABA concentration than Williams-82 at 48 h (the critical stage) and 192 h (at nodule initiation) after inoculation.

Uptake and Distribution of ^3H -ABA

There was no significant difference in the two cultivars in the uptake of radioactivity (Figure 5) from leaf applied ^3H -ABA. However, the inoculation increased the percentage uptake in NODI-3 while Williams-82 showed the opposite trend. The re-

tention of radioactivity from ^3H -ABA was greater in leaves of both uninoculated and inoculated plants of NODI-3 as compared to Williams-82. The difference between the two lines was greater between 48 and 72 h after inoculation (Figure 6a-d). The percentage distribution of radioactivity from ^3H -ABA was greater in the root and phloem sap of the inoculated Williams-82 parent, particularly 48-72 h after inoculation. The

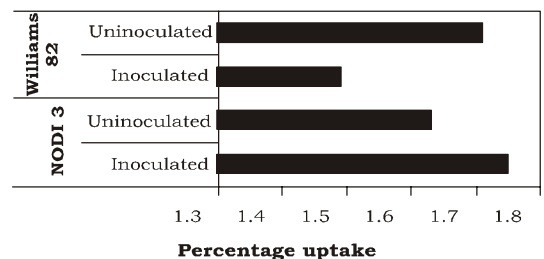


Figure 5. Percentage uptake by whole plant in normal and hypernodulating soybean cultivars

UPTAKE AND DISTRIBUTION PATTERN OF APPLIED ABSCISIC

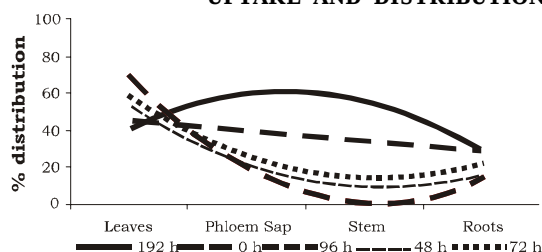


Figure 6a. Distribution pattern of ^3H -ABA in inoculated plants of soybean cv. NODI-3 from the time of inoculation and ^3H -ABA application to root nodule initiation

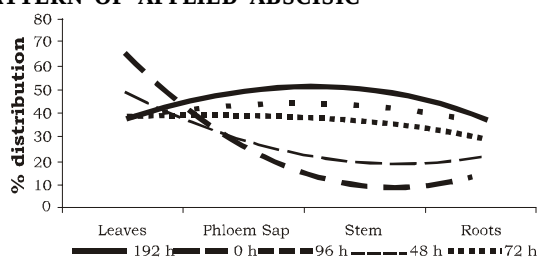


Figure 6c. Distribution pattern of ^3H -ABA in uninoculated plants of soybean cv. NODI-3 from the time of inoculation and ^3H -ABA application to root nodule initiation

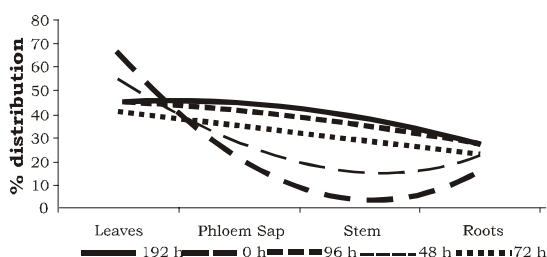


Figure 6b. Distribution pattern of ^3H -ABA in inoculated plants of soybean cv. Williams-82 from the time of inoculation and ^3H -ABA application to root nodule initiation

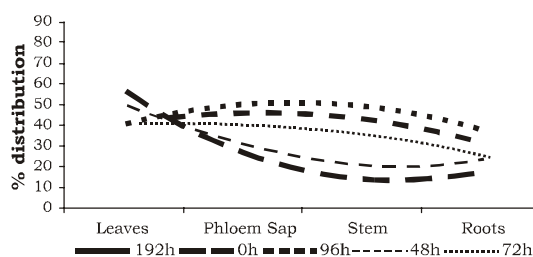


Figure 6d. Distribution pattern of ^3H -ABA in uninoculated plants of soybean cv. Williams-82 from the time of inoculation and ^3H -ABA application to root nodule initiation

roots of both the cultivars accumulated less radioactivity from ^3H -ABA than that of stem. The uninoculated plant roots of both the cultivars accumulated ABA greater than inoculated ones. Suzuki et al. (2004) indicated that lower-than-normal concentrations of endogenous ABA enhance nodule formation and it has been hypothesized that the ABA concentration controls the number of root nodules.

The percentage distribution of radioactivity in the phloem sap was greater in both the uninoculated and inoculated plants of Williams-82 compared to NODI-3. The observed increase of ABA in phloem sap of NODI-3 was particularly evident at 48-72 h after inoculation, and this may be indicative of an adaptive mechanism in NODI-3 that is possibly required for auto regulation of root nodulation. At nodule initiation, the marked decrease in the ABA concentration in leaf of the hypernodulating mutant (NODI-3), as compared to that of its nor-

mally nodulated parent (Williams-82) that showed higher levels of ABA at nodule initiation (192 h after inoculation), is indicative of the negative role of ABA in nodule morphogenesis.

The age related changes in the ABA level of the two cultivars demonstrated that exogenous application of ABA at the nodule primordia stage arrested the growth and development of the nodules (Liang et al., 2007).

The data indicated a marked redistribution of radioactivity from leaves to other parts. The maximum distribution percentage of radioactivity from leaf applied ^3H -ABA was in phloem sap, which indicates that ABA may be the inhibitor moving through the phloem and transported from the leaf. The rate of transport of radiolabelled ABA from the leaf suggested that there are slow rates of transport of ABA in NODI-3.

It is inferred that the phloem sap of NODI-3 contained lower ABA concentration

from 48h to 72h after inoculation than did Williams-82. It is also speculated that the difference between the two lines in the concentration of ABA of leaf and phloem, as evidenced by transport and distribution of radiolabelled ABA, was most obvious at 48 h after inoculation, which coincides with the perceived critical phase for onset of auto regulation.

LITERATURE CITED

- Bano, A. and Hillman, J. R. 1989. Uptake, distribution and metabolism of (2-¹⁴C) Absciscic acid in *Faba vulgaris* (*Vicia faba*) and *Alnus glutinosa*. Crop Res. (Hort Res). 29: 77-86.
- Cooper, J.B. and Long, S.R. 1994. Morphogenetic rescue of *Rhizobium meliloti* nodulation mutants by trans-zeatin secretion. The Plant Cell, 6: 215-225.
- Delrot, S. A. Everat-Bousbouloux, A. and Bonnemain, L. 1981. Effect of pH on the uptake and export of abscisic acid by leaf tissue of *Vicia faba* C.R.Acad.Sci.D. 293: 659-663.
- Delves, A. C. Mathews, A. Day, D. A. Carter, A. S. Carroll, B. J. and Gresshoff, P. M. 1986. Regulation of the soybean-Rhizobium symbiosis by shoot and root factors. Plant Physiol. 82: 588-590.
- Ding, Y. and Oldroyd, G.E.D. 2009. Positioning the nodule hormone doctum. Plant Signal Behav. 4(2): 89-93.
- Hirsch, A. M. 1992. Development biology of legume nodulation. New Phytol. 122:211-237.
- Hirsch, A.M. and Fang, Y. 1994. Plant hormones and nodulation: what's the connection? Plant Mol. Biol. 26: 5-9.
- Hirsch, A. M. Fang, Y. Asad, S. and Kapulnik, Y. 1997. The role of phytohormones in the plant-microbe symbiosis. Plant Soil. 194:171-184.
- Liang, Y. Mitchell, D. M. and Harris, J. M. 2007. Absciscic acid rescues the root meristem defects of the *Medicago truncatula* latd mutant. Dev Biol. 304:297-307
- Oldroyd, G.E. and Downie, J. A. 2008. Coordinating nodule morphogenesis with rhizobial infection in legumes. Ann. Rev. Plant Biol. 59:519-546.
- Sheng, C. and Harper, J. E. 1997. Shoot verses root signals involvement in nodulation and vegetative growth in wild-type and hypernodulating soybean genotypes. Plant Physiol. 13:825-831.
- Suzuki, A. M. Akune, M. Kogiso, Y. Imagama, K. Osuki, T. Uchiumi, S. Higashi, S.Y. Han, S. Yoshida, T. A. and Abe, M. 2004. Control of nodule number by the phytohormone abscisic acid in the roots of two leguminous species. Plant Cell Physiol. 45:914-922.
- Watts, S. H. Wheeler, C. T. Hillman, J. R. Barrie, M. M. Crozier, A. and Math, V. B. 1983. Absciscic acid in the nodulated root system of *Alnus glutinosa*. New Phytol. 95: 203-208.