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Foliar application of pyraclostrobin fungicide enhances the growth, rhizobial-nodule formation and nitrogenase activity in soybean (var. JS-335)

Juhie Joshi *, Sonika Sharma, K.N. Guruprasad

School of Life Sciences, Devi Ahilya University, Khandwa Road, Indore, M.P. 452001, India

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ABSTRACT

A field study was conducted to investigate the impact of the fungicide pyraclostrobin (F500 – Headline®; a.i. 20%) on the activity of nitrogenase in soybean (var. JS-335). Pyraclostrobin (F500) was applied on the leaves of soybean plants at 10 and 20 days after emergence (DAE) of seedlings at concentrations ranging from 0.05% to 1%. Leghemoglobin (Lb) content and nitrogenase activity in root nodules were analyzed at 45th day after emergence of seedlings indicated a remarkable increase in Lb content and enhanced activity of nitrogenase in the root nodules of pyraclostrobin treated plants. The fungicide also enhanced the number of nodules along with weight of nodules, root biomass and growth of shoot and leaves. Enhanced nitrogen fixation in the root nodules by pyraclostrobin improves the growth of the plant in soybean before flowering and pod formation which ultimately resulted in yield and yield attributes. These results suggest that pyraclostrobin (F500) can be successfully employed as a foliar spray under field conditions to enhance the growth, nitrogen assimilation and hence yield of soybean.

1. Introduction

Pyraclostrobin (F500 – Headline®), a strobilurin class of fungicide was initially developed by BASF as a broad spectrum fungicide with preventive, curative, translaminar and locosystemic properties. Pyraclostrobin is a fungicide of strobilurins group and it acts by inhibiting mitochondrial respiration by blocking the transfer of electrons in the III complex (bc1 complex) of the transporting current for mitochondrial electrons [1]. Strobilurin is produced by a basidiomycetes wood rotting fungus, *Strobilurus tenacellus* (Pers.) Singer [2]. Few earlier studies have revealed the non-fungicidal physiological effects of pyraclostrobin on crop plants. Cereals treated with pyraclostrobin show significant increases in production, greater than those due only to its fungicidal effect [3]. Change in hormonal balance, water conservation and delayed senescence are some of the positive physiological changes observed after the application of strobilurin, which have been related to higher yield [4]. Wheat plants treated with strobilurin produced more yields [5,6].

Soybean (*Glycine max* (L.) Merr.) is a grain legume of considerable dietetic, industrial, medicinal and economic importance [7,8]. India is the fifth largest producer of soybean in the world and as an oilseed crop it is only next to groundnut and mustard.

Soybean as a legume possesses "nodules" which are unique, specialized organs that are the result of a symbiotic association between plants of the family *Leguminosae* and soil bacteria of the genera *Sinorhizobium*, *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium*. The bacteria are present in the infected plant cell surrounded by the peribacteroid membrane, which is derived from the plant cell membrane. There they differentiate into bacteroids and express the enzyme nitrogenase, which enables them to fix atmospheric dinitrogen, thus allowing the plant host to grow without external reduced nitrogen. Nitrogenase is oxygen-sensitive, but the vigorously respiring bacteroids require an adequate supply of oxygen. This is achieved by the presence of leghemoglobin, which facilitates oxygen diffusion to the endosymbiont. Leghemoglobin has been immunolocalized to the cytosol of the infected plant cell, and is absent from the bacteroid and peribacteroid space [9].

Growth of soybean is enhanced by foliar spray of pyraclostrobin [10]. Application of pyraclostrobin enhanced rate of photosynthesis and leaf nitrate reductase activity in soybean that resulted in enhancement in yield [11]. Begliomini et al. [12] also reported similar results on soybean observing in addition reduced ethylene production and reduced rate of respiration after the application of strobilurin. Strobilurin treatment enhanced nitrate assimilation in wheat plants [5,6].

* Corresponding author. Fax: +91 01202701613.
E-mail address: juhiejoshi@yahoo.com (J. Joshi).

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To best of our knowledge this is the first report for evidence of enhancement in nitrogen fixation by nitrogenase enzyme by the application of strobilurin. The previous studies indicating enhancement in carbon and nitrogen assimilation by the application of strobilurin have focused on the rate of photosynthesis and nitrate reductase activity in the leaves. There is no data on the impact of strobilurin on the activity of root nodules of the legumes. The aim of the present investigation was to study the impact of foliar application of strobilurin on the activity of the nitrogenase enzyme that can directly fix nitrogen from the atmosphere in soybean.

2. Materials and methods

2.1. Plant material and treatments

Seeds of soybean var. JS-335 were collected from Directorate of Soybean Research, Indore (M.P.), India. Field experiments under natural sunlight were conducted in the botanical garden of School of Life Sciences, Indore (22.4°N), India. Seeds were inoculated with slurry of *Rhizobium japonicum* at 3 g/kg seeds before sowing. The plots were watered as needed and weeds were controlled manually.

The seeds were sown in plastic bags (34 cm H × 34 cm B; filled with mixture of sand, soil and manure −1:4:1) and kept under field conditions. The experiments were carried out in August 2011 to October 2011 and September 2011 to November 2011. Pyraclostrobin was sprayed on the leaves at 10 and 20 DAE. Samples were taken at an interval of 10 days after 35th DAE for collecting the growth data, number of nodules and weight per nodule. Each experiment was done in triplicates of five plants each. Disease-free plants were used in our experiments and no disease was detected in untreated plants during our field experiments.

2.2. Chemical details

Common name: Pyraclostrobin. Exp. Code Numbers: F 500 (BASF). Labeled usage rate: applied at a rate of 500 g formulation per ha. A formulation of F 500 (BAS 500 F DI) containing 20% (w/w) active ingredient was provided by BASF Inc. (Limburgerhof, Germany).

2.2.1. Composition

Methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy-methyl}-phenyl)N-methoxycarbamate (IUPAC); Carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester(CAS)

Class: Strobilurin.

Molecular formula: $C_{19}H_{18}N_3O_4Cl$.

2.2.2. Foliar application of F500

Pyraclostrobin (F500) was sprayed on 10th and 20th day after emergence of seedlings (DAE) using 0.05%, 0.1%, 0.25%, 0.5%, 1% concentrations and unsprayed plants served as control.

2.3. Growth analysis

Plant height, leaf area, root length (length of longest root), root weight, number of nodules per plant and weight of nodules were measured at an interval of 10 days till 65 DAE and leaf area was monitored in fully expanded third leaf from the top. Area of the leaves was measured using portable laser leaf area meter CI-202 scanning planimeter (CID Inc., USA). Flowering was initiated at 45 DAE.

2.4. Extraction and estimation of leghemoglobin (Lb) content

Leghemoglobin (Lb) was extracted from the root nodules of 45 day old soybean plants and measured by the method of Jun et al. [13] Root nodules (1.25 g) were crushed in liquid nitrogen in a mortar with pestle. The resulting powder was resuspended in 25 mL of 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM EDTA, 1 mM PMSF, β-mercaptoethanol and 10% polyvinyl

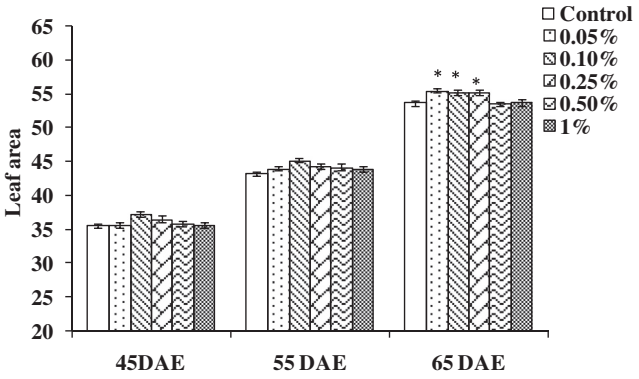

Fig. 2. Effects of foliar spray of pyraclostrobin (F500) on leaf area of soybean at different concentrations of F500 at 45th, 55th and 65th DAE. The vertical bar indicates ± SEM for mean. Values are significantly different at $^{*}P < 0.05$ from untreated plants (Newman–Keuls Multiple Comparison Test).

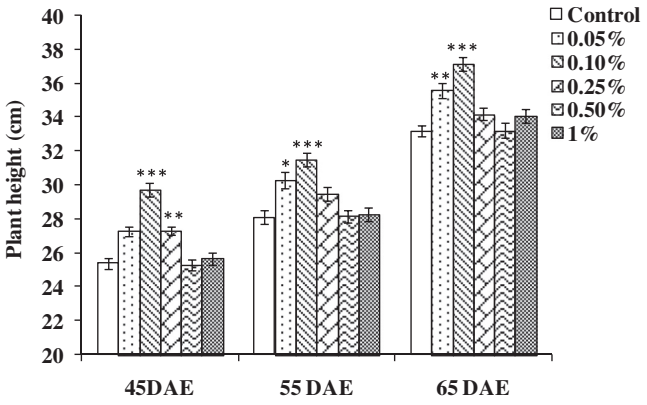

Fig. 1. Effects of foliar spray of pyraclostrobin (F500) on plant height of soybean at different concentrations of F500 at 45th, 55th and 65th DAE. The vertical bar indicates ± SEM for mean. Values are significantly different at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ from untreated plants (Newman–Keuls Multiple Comparison Test).

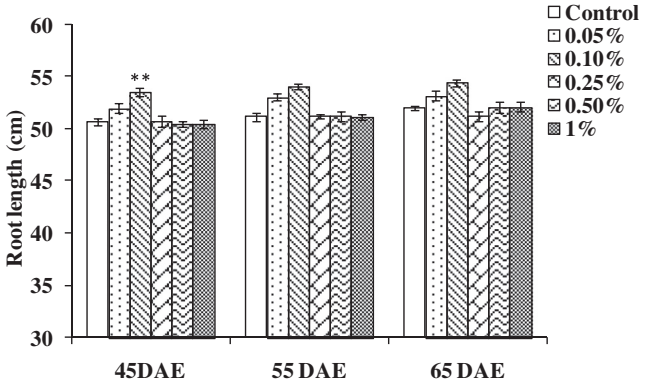

Fig. 3. Effects of foliar spray of pyraclostrobin (F500) on root length of soybean at different concentrations of F500 at 45th, 55th and 65th DAE. The vertical bar indicates ± SEM for mean. Values are significantly different at $^{**}P < 0.01$ from untreated plants (Newman–Keuls Multiple Comparison Test).