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COMBINED EFFECTS OF SOME FERTILIZERS AND PHYTOHORMONES ON NITRATE REDUCTASE ACTIVITY AND SOLUBLE PROTEIN IN THE LEAVES OF GREEN-GRAM [*VIGNA RADIATA* (L.) WILCZEK]

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Field experiments were conducted to study the combined effects of some fertilizers, *viz.*, $(NH_4)_2SO_4$, KH_2PO_4, KCl and $MgSO_4.7\ H_2O$ and phytohormones (IAA, GA_3 and kinetin) on nitrate reductase activity and soluble protein in the leaves of green-gram. Foliar spray treatments of each salt or PGRs increased the *in vivo* nitrate reductase (NR) activity as well as the level of soluble protein over the control (water spray). The combined foliar spray of each salt with PGRs (IAA, GA_3 and kinetin) exhibited positive interaction in terms of enzyme activity.

Keywords : Fertilizer & Phytohormones effect; *in vivo;* Nitrate reductase activity; Soluble protein; *Vigna radiata.*

Introduction

Mineral nutrients constitute an important ingredient of intensive farming. Essentially the use of mineral nutrients for achieving high biomass has been reported in many plants by a number of workers[1-10].

Nitrate is the predominant form of inorganic nitrogen available to the plants for their growth and development. Nitrate reductase (NR) is the first enzyme of nitrate assimilation pathway. NR (E.C.1.6.6.1) is a rate limiting and substrate inducible enzyme and its activity is positively correlated with total protein, organic nitrogen content and sometimes overall productivity of plants[11-12]. Besides nitrate, other nitrogenous metabolites such as ammonium, amino acids and amides are also known to influence the activity of NR[13]. In higher plants the inducible nature of nitrate reductase has been well established[14]. Field response of leguminous plants to inoculation and fertilizer application has been reviewed by Subba Rao[15].

In the recent years the foliar application of plant growth regulators on crop plants have been used to enhanced plant growth, development and yield[16-24]. Plant growth regulators are known to regulate flowering, fruit setting and fruit development. Appilication of GA_3 promoted or induced flower initiation in many long-day plants[25].

In the present investigation combined effects of three non-nitrogenous fertilizers (KH_2PO_4, KCl and $MgSO_4.7\ H_2O$) and a nitrogenous fertilizer (ammonium sulphate) with three plant growth regulators (IAA, GA_3, and KIN) in the form of leaf treatment, were studied on the *in vivo* NR activity and soluble protein.

Materials and Methods

Plant growth- Seeds of *Vigna radiata* (L.) Wilczek, cv Cultivated variety K 851 were obtained from National Seeds Corporation, IARI, Pusa, New Delhi. Surface sterilized seeds (with 95% ethanol for 2 min) were thoroughly washed with distilled water and soaked with distilled water for 12 h in an incubator at 30 ± 2° C. The soaked seeds were sown in garden soil. After germination the seedlings were thinned out and only 10 seedlings were allowed to grow in each plot of 1 m x 1 m size.

Foliar application of salts and growth regulators- In all cases optimum concentrations 5 mM for each salt and 10 µg/ml for each PGR were used, as determined by our previous experiments. The trial for combination treatment experiment consisted of ten sets in addition to a control set (water spray). The treatments included—optimum concentration of each salt (T_1), i.e., 5 mM $(NH_4)_2SO_4$, 5 mM KH_2PO_4, 5 mM KCl and 5 mM $MgSO_4.7\ H_2O$, optimum concentration of each PGR, i.e., 10 µg/ml IAA (T_2), 10 µg/ml GA_3 (T_3) and 10 µg/ml kinetin (T_4), optimum concentration of each salt + optimum concentration of IAA (T_5), optimum concentration of each salt + optimum concentration of GA_3 (T_6), optimum concentration of each salt + optimum concentration of kinetin (T_7), optimum concentration of IAA + GA_3 + kinetin (T_8) and optimum concentration of each salt + optimum concentration of IAA + GA_3 + kinetin (T_9). In each combination the optimum concentration of each salt and PGR was maintained in the final volume. The control plants were sprayed with water. Foliar spray of different combinations of salts and hormones were applied to the 15-day-old plants with the help of a hand sprayer.

In vivo NR activity the *in vivo NR activity* was measured in fresh leaves on zero day, i.e., just before spray treatment and continued at weekly intervals for three weeks in the control and sprayed plants. The NR activity was determined by the method of Hageman and Hucklesby[26]. *Phosphate-buffer soluble protein*- Soluble protein was estimated in the fresh leaves on zero day, *i.e.*, just before spray and onward up to three weeks at weekly interval by following the method of Lowry *et al.*[27]. The protein from the fresh tissue was extracted from leaves in phosphate-buffer (0.1 M; pH 7.5). In each case it was precipitated out with equal volume of 10% trichloro acetic acid. Bovine serum albumin was used as a standard.

Results and Discussion

In the present study foliar spray of some fertilizers and phytohormones alone and in combination enhanced the rates of *in vivo* NR activity and soluble protein over the control (water spray). Foliar spray of optimum concentration of $(NH_4)_2SO_4$ and each phytohormone (IAA, GA_3 and kinetin) alone and in combination, *viz*., $(NH_4)_2SO_4$ + IAA, $(NH_4)_2SO_4$ +GA_3, $(NH_4)_2SO_4$ + kinetin, IAA +GA_3 +kinetin and $(NH_4)_2SO_4$ + IAA +GA_3 + kinetin enhanced the rates of NR activity over control (water spray). The most effective combination was found to be 5 mM $(NH_4)_2SO_4$+ 10 μg/ml GA_3 on day 14 after spray. The data is shown in the Fig. 1A.

Foliar spray of optimum concentration of KH_2PO_4 and each phytohormone (IAA, GA_3 and kinetin) alone and in combination, *viz*., KH_2PO_4+ IAA, KH_2PO_4+GA_3, KH_2PO_4+ kinetin, IAA +GA_3 +kinetin and KH_2PO_4+ IAA +GA_3 + kinetin enhanced the rates of NR activity over control (water spray). With 5 mM KH_2PO_4 the NR activity was higher than any other treatment on the 7th and 14th days after spray. The NR activity was higher on day 21 after spray with each treatment of PGR in combination with KH_2PO_4.The most effective combination was found to be 5 mM KH_2PO_4+ 10 μg/ml kinetin on day 21 after spray. Nitrate reductase activity was higher with optimum concentration of KH_2PO_4 in combination with hormones than application of KH_2PO_4 alone. The data is shown in the Fig. 1B.

Foliar spray of optimum concentration of KCl and each phytohormone (IAA, GA_3 and kinetin) alone and in combination, *viz*., KCl+ IAA, KCl+GA_3, KCl+ kinetin, IAA +GA_3 +kinetin and KCl+ IAA +GA_3 + kinetin enhanced the rates of NR activity over control (water spray). The most effective combination was found to be 5 mM KCl+ 10 μg/ml GA_3 on day 7after spray. On day 21 after spray treatment with 5 mM KCl + 10 μg/ml kinetin was most effective in increasing NR activity. The data is shown in the Fig. 1C. The stimulation of nitrate redutase activity of potassium salts is generally in agreement with the response reported earlier by different workers in a number of plants[14,28,29]. K^+ ions have been shown to affect nitrate reductase in various ways[14,30,31]. Sharma and Agarwal[32] observed that potassium treatment increased nitrate reductase activity in *Cicer arietinum.*

Foliar spray of optimum concentration of $MgSO_4.7H_2O$ and each phytohormone (IAA, GA_3 and kinetin) alone and in combination, *viz*., $MgSO_4.7H_2O$+ IAA, $MgSO_4.7H_2O$+GA_3, $MgSO_4.7H_2O$+ kinetin, IAA +GA_3 +kinetin and $MgSO_4.7H_2O$ + IAA +GA_3 + kinetin enhanced the rates of NR activity over control (water spray). The most effective combination was found to be 5 mM $MgSO_4.7H_2O$+ 10 μg/ml IAA + 10 μg/ml GA_3 + 10 μg/ml kinetin on day 21 after spray. The data is shown in the Fig. 1D.

Singh and Chaudhuri[7] showed that application of S, along with K and P in the soil, increased the nodulation, podding and pod and haulm yield of groundnut. Pandey and Venu Babu[2] observed that application of phosphorus @ 60 kg P_2O_5/ha increased the activity of NR and GDH while lower dose (30 kg P_2O_5/ha) was most favourable for GS activity. Phosphorus @ 80 kg P_2O_5/ha decreased the enzyme activity. Magnesium is essential to the plants because of its presence in the chlorophyll. Mg^{2+} also activates many enzymatic reactions that involve ATP such as photosynthesis, respiration and formation of DNA and RNA[33].

Nitrogen is a very important nutrient element in agriculture. In soil it occurs in organic and inorganic forms[34]. Within the integrated multiple cropping system nitrogen is the most common limiting nutrient needed to increase agricultural production. This nutrient can be obtained at high cost from commercial fertilizers or through biological nitrogen fixation by leguminous plants. Foliar spray of optimum concentration of $(NH_4)_2SO_4$ and each phytohormone (IAA, GA_3 and kinetin) alone and in combination, *viz*., $(NH_4)_2SO_4$ + IAA, $(NH_4)_2SO_4$ +GA_3, $(NH_4)_2SO_4$ + kinetin, IAA +GA_3 +kinetin and $(NH_4)_2SO_4$ + IAA +GA_3 + kinetin enhanced the level of soluble protein over control (water spray). The level of soluble protein was higher on day 7 after spray with treatment of each PGR in combination with 5 mM $(NH_4)_2SO_4$. However, the most effective combination was found to be *5 mM* $(NH_4)_2SO_4$ + *10 μg/ml* GA_3 *on day 14* after spray. On day 21 after spray maximum level of soluble protein was obtained in the combination containing mixture of each PGR. The data is shown in the Fig. 2A.

Foliar spray of optimum concentration of KH_2PO_4 and each phytohormone (IAA, GA_3 and kinetin) alone and in combination, *viz*., KH_2PO_4+ IAA, KH_2PO_4+GA_3, KH_2PO_4+ kinetin, IAA +GA_3 +kinetin and KH_2PO_4+ IAA +GA_3 + kinetin enhanced the level of soluble protein over

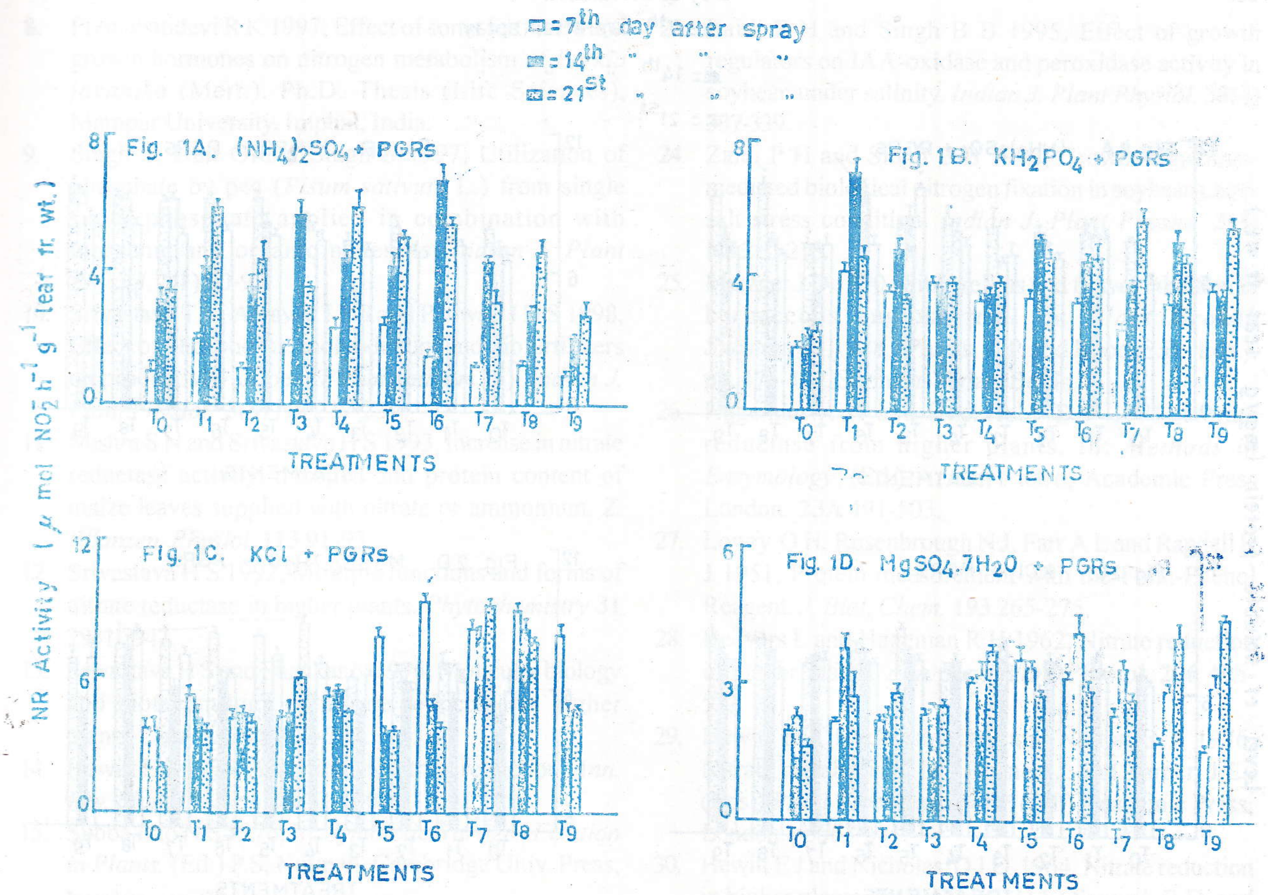

Fig. 1. *In vivo* nitrate reductase (NR) activity on different days in the leaves of *Vigna radiata* sprayed with optimum concentration (5 mM) of each salt or each PGR (10 μg/ml) and their various combinations. T_0 = control, T_1 = each salt (5 mM), T_2 = IAA (10 μg/ml), T_3 = GA_3 (10 μg/ml), T_4 = kinetin (10 μg/ml), $T_5 = T_1+T_2$, $T_6 = T_1+T_3$, $T_7 = T_1+T_4$, $T_8 = T_2+T_3+T_4$, $T_9 = T_1+T_2+T_3+T_4$.

control (water spray). The level of soluble protein was higher on all days with treatments of each PGR in combination with 5 mM KH_2PO_4. The most effective combination was found to be 5 mM KH_2PO_4 + 10 μg/ml IAA + 10 μg/ml + GA_3 +10 μg/ml kinetin on day 7, 14 and 21 after spray (Fig. 2B).

Foliar spray of optimum concentration of KCl and each phytohormone (IAA, GA_3 and kinetin) alone and in combination, *viz.*, KCl + IAA, KCl+GA_3, KCl + kinetin, IAA +GA_3 +kinetin and KCl + IAA +GA_3 + kinetin enhanced the level of soluble protein over control (water spray). The level of soluble protein was higher on all days with treatment of each PGR in combination with 5 mM KCl. The most effective combination was found to be 5 mM KCl + 10 μg/ml IAA on day 7, 14 and 21 after spray. The data is shown in the Fig. 2 C.

Foliar spray of optimum concentration of $MgSO_4.7H_2O$ and each phytohormone (IAA, GA_3 and kinetin) alone and in combination, *viz.*, $MgSO_4.7H_2O$ + IAA, $MgSO_4.7H_2O$+GA_3, $MgSO_4.7H_2O$ + kinetin, IAA +GA_3 +kinetin and $MgSO_4.7H_2O$ + IAA +GA_3 + kinetin enhanced the level of soluble protein over control (water spray). The most effective combination was found to be 5 mM $MgSO_4.7H_2O$ + 10 μg/ml GA_3 on day 21 after spray. The data is shown in the Fig. 2 D.

A number of workers studied the effect of different plant growth substances, *viz.*, IAA, GA_3, KIN, NAA, 6-benzylamino-purine (BAP), Coumarin (COU), malic hydrazine (MH), 2,3,5-triiodobenzoic acid (TIBA) and CCC on growth and nodule development of leguminous plants such as *Pisum sativum, Physalis peruviana, P. angulata* and *Vicia faba*[35-37]. Premabatidevi[38] observed increase in the activity of *in vivo* nitrate reductase and nitrite reductase in the leaves of a tree legume-*Parkia javanika* with spray of IAA, GA_3 and KIN.

Further, it was noticed that the level of soluble

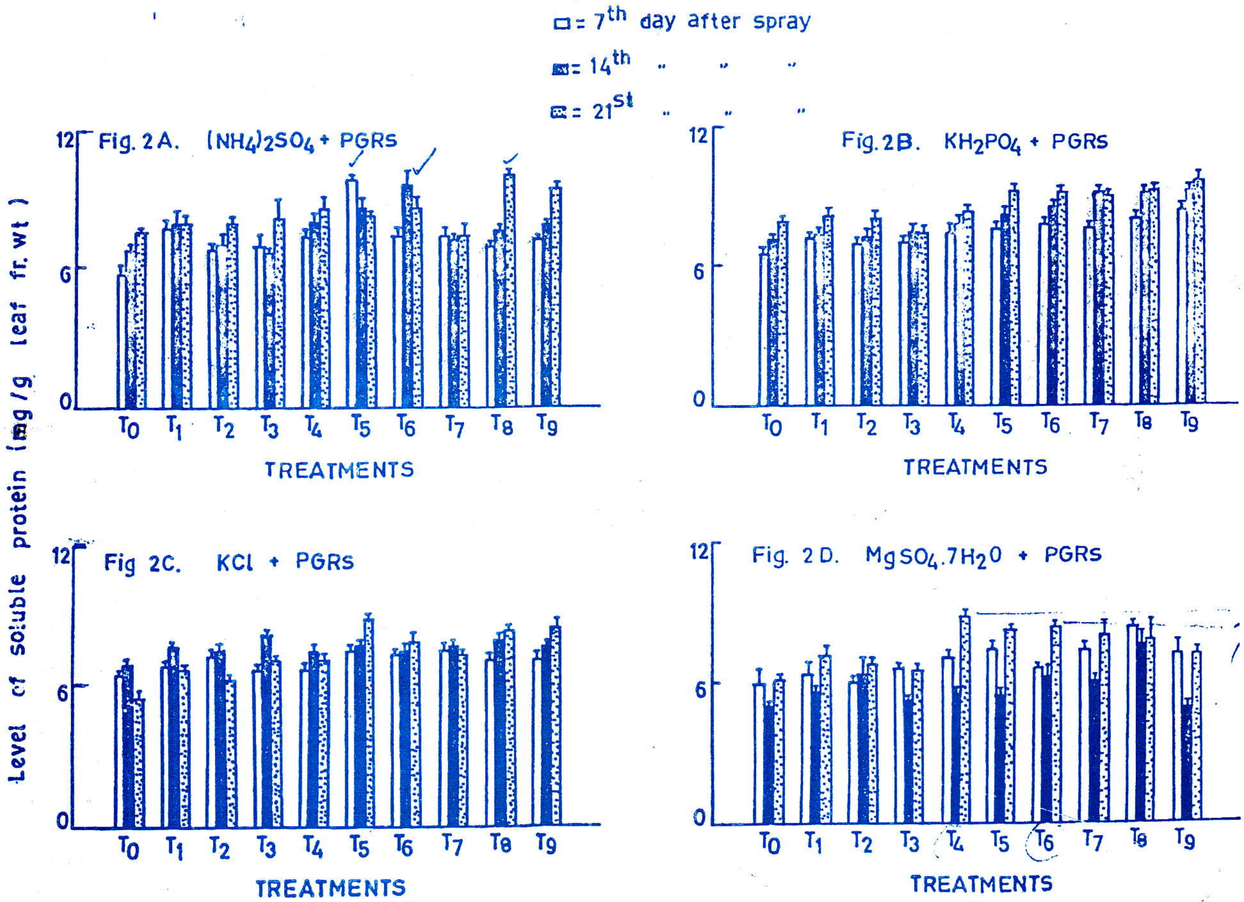

Fig. 2. Level of soluble protein on different days in the leaves of *Vigna radiata* sprayed with optimum concentration (5 mM) of each salt or each PGR (10 μg/ml) and their various combinations. T_0 = control, T_1 = each salt (5 mM), T_2 = IAA (10 μg/ml), T_3 = GA_3 (10 μg/ml), T_4 = kinetin (10 μg/ml), $T_5 = T_1 + T_2$, $T_6 = T_1 + T_3$, $T_7 = T_1 + T_4$, $T_8 = T_2 + T_3 + T_4$, $T_9 = T_1 + T_2 + T_3 + T_4$.

protein was higher in those tretment sets where NR was also higher and *vice versa*. Thus, *in vivo* NR can be taken as an index of higher soluble protein level in the green-gram leaves. In this regard Johnson *et al.*[39] suggested that NR is a parameter ultimately determines yield or leaf biomass. Therefore, it is expected that the treatments that increased NR activity also enhance the crop yield.

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