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Impact of phosphorous on biochemical changes in *Hordeum vulgare* L. in mixed cropping with Chickpea

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Abstract: *Multiple cropping (i.e. intercropping or mixed cropping) plays an important role in agriculture because of the effective utilization of resources, significantly enhancing crop productivity compared with that of monocultured crops. The study was planed to assess the effect of various concentrations (00, 30, 60, 90 kg ha^{-1}) of phosphorous on the biochemical composition of grains of Hordeum vulgare L. (NDB-1050) in mixed cropping system with Chickpea. Phosphorous is an essential ingredient for plants to convert atmospheric N (N_2) into an ammonium (NH_4) as a useable form. The available nitrogen content was found more in the year 2006 (131 kg ha^{-1}) than year 2005 (105 kg ha^{-1}). The results of available nitrogen content were showed that the mixed cropping system enhances N fixation process because phosphorous also influences nodule development through its basic functions in plants as an energy source. Reducing, non reducing and total sugar content of H. vulgare L. were influenced by changes in the phosphorous doses. Maximum protein (13.43 %) was obtained at 60 kg P_2O_5 ha^{-1} during the year 2006. Lysine, tryptophan and methionine content were found maximum in year 2006, respectively. Total mineral content of grains of plant (0.99 g 100g^{-1}) was found maximum by the application of 60 kg P_2O_5 ha^{-1}. It is possible that there was an increase in the soil N made available by the leguminous chickpea species, and this could be another reason why there was an increase in Hordeum vulgare L. shoot mass per plant with intercropping with chickpea.*

Key words: *Cropping system, Nitrogen fixation, Legume, Nutrition, Phosphorous*
PDF of full length paper is available online

Introduction

Phosphorous plays a vital role as a structural component of cell constituent and metabolically active compounds i.e. chloroplasts, mitochondria, phytin, nucleic acid, protein, flavin nucleotides and several enzymes. It also plays a crucial role in growth and development of roots, tillers and grains, energy transformation and metabolic process of plants (Wang *et al.*, 2006). Adequate level of phosphorous in soil is essential for nitrogen uptake (Schippers *et al.*, 2006) and if the phosphorous become limiting to plants do not grow normally, yield and quality of grains is consequently reduced (Sirkar *et al.*, 2000). Malnutrition and micronutrient deficiencies (especially Fe and Zn) can prevalent in many chickpea and wheat consuming region, even though chickpea and wheat seeds are good sources of essential mineral nutrients (Wang *et al.*, 2003). Multiple cropping (*i.e.* intercropping or mixed cropping) plays an important role in agriculture because of the effective utilization of resources, significantly enhancing crop productivity compared with that of monocultured crops (Li *et al.*, 1999). Interspecific competition and facilitative interactions which may occur when two crops are grown together have been extensively investigated (Li *et al.*, 2004), and are attracting increasing interest.

The current trend in global agriculture is to search for highly productive, sustainable and environment friendly cropping systems (Crew and Peoples, 2004). The specific plant responses vary with many factors including plant species and genotype, age of plants, time and tissue type (Sharma *et al.*, 2005). Prolong phosphorous exposure can have striking effects on the composition and quantity of proteins and can increase or decrease the activities of the vital enzymes involved in synthesis (Subbaiah and Sachs, 2003).The amount of acid phosphatase secreted by plants is genetically controlled and differs with crop species and varieties as well as crop management practices (Wright and Reddy, 2001). Some studies have shown that the amount of enzymes secreted by legumes were 72% higher than those from cereals (Yadav and Tarafdar, 2001). However, there is prevailing hypothesis about the role of phosphates in plants and its relation to plant nutritional status i.e. plants adapted to Pi stress would present high leaf or root P-ase activity as a sign of hydrolyzing and remobilizing Pi, by root secretion and leaf synthesis, making Pi more available to plants, from soil or plant parts (Sharma *et al.*, 2005; Nielsen and Jensen, 2005). *H. vulgare* is an important cereal crop used in religious ceremonies in India. Out of total production about 80% is used as a food material, 10% as an animal feed and 10% as a raw material for manufacturing of alcoholic products. Its grains have good nutritive value and contain 10-11.5% protein (but in case of high protein variety12-20% protein), 74% carbohydrate, 1.3% fat, 3.9% crude fiber, 1.5% ash and 1.2% minerals. It has been reported *H. vulgare* grain yield increased with seed placed P+N in small amount of addition to traditional band placed fertilizer (Wang *et al.*, 1998; Li *et al.*, 2001). Therefore the present study was planned on phosphorous related effects on biochemical composition of *H. vulgare* grain quality (Ghotra *et al.*,

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2007). The objective of this study was to assess the effects of different phosphorous levels on biochemical composition of *H. vulgare* L. in mixed cropping system with chickpea.

Materials and Methods

Plant material: The plant of *H. vulgare* (Narendra Dev Barley = NDB-1050) used as an experimental material to grow in a mixed cropping system with Chickpea (*C. arietinum)* in the farm of National Environmental Engineering Research Institute, Nagpur. This farm received an annual rainfall about 1270 mm with relative humidity of 55-100 %. During the summer, temperature is ranged from 25-45°C and in winter 4-25°C. Experiments were setup with breeder seed of plant in a complete randomized design with three replication. The row-to-row and plant-to-plant spacing was 15 cm; and the seed used @ 80 kg ha^{-1} in case of *H. vulgare* and @100 kg ha^{-1} in *C. arietinum.* Fertilizers (IFFCO, India), half dose of nitrogen and potash were applied @ 50:20 kg ha^{-1}, respectively. The applied phosphorous was 0, 30, 60 and 90 kg ha^{-1} in the field. Half dose of nitrogen and full dose of potash were applied as basal application whereas; remaining ½ dose of nitrogen was top-dressed after first irrigation. Besides, the crop was grown with the recommended package of agronomic practices to achieve good response.

Biochemical analysis: Available nitrogen was determined before sowing of crop by the alkaline permanganate method. The powdered plant grains were used for the determination of biochemical parameters. The sample was dried at 10°C for 6-8 hr and 100 mg was transferred to test tube with the addition of 10 ml ethanol (50%). The upper layer is collected and adds 10 ml distilled water. This extract was used for estimation of reducing sugar and total sugar by dinitrocyclic acid reagent method described by. The reducing sugar content of barley flour was estimated by dinitro salicyclic acid (DNS) reagent method (Miller, 1959). The sugar extract (2.0 ml) of prepared extract was taken in the test tube and 3.0 ml DNS reagent was added. It was kept on boiling water bath for 5.0 minutes. Further, it was cooled at room temperature and the intensity of color was recorded at 575 nm on a spectrophotometer (Spectronic 20). The non reducing sugar was obtained by substruction of reducing sugar from total sugar. The total sugar content was determined by spectrophotometer (Dubois *et al.,* 1956), which allows the determination of the total sugar even in very small quantities. A Varian Spectrophotometer (Spectronic-20) was used for the spectrophotometric readings. Ten readings at 490 nm were taken 45 minutes after the brown color resulting solution, using a 1 cm optical path glass cuvette.

The procedure of protein estimation was based on method of Lowry *et al.* (1951) from the reaction of protein with egg albumin as reference. The procedure was based on quantitating the color obtained from the reaction of protein with folinciocalteau reagent. One gram barley flour was taken and homogenized in 10.0 ml double distilled water. The whole content was finally centrifuged at 4000.0 rpm for 15 minutes. Thereafter, 1.0 ml supernatant was taken and mixed with 1.0 ml TCA (10%). It was kept to 30 minutes and the residue obtained was dissolve in 5.0 ml 0.1 N NaOH. Further, 5.0 ml alkaline copper reagent was added to the same in test tube and mixed properly. After 10 minutes, 0.5 ml folin reagent was added and kept at room temperature for 30 minutes. Finally intensity of color was recorded at 660 nm at spectrophotometer (Spectronic-20) against the blank solution.

Methionine content was analyzed following by Horn *et al.*, (1949). 0.5 g sample was weighed and transferred to a flask. 20.0 ml 6.0 N HCl was added in the samples. The material was reflexed for 20 to 24 hr then transfered into china disk. It was evaporated on water bath with the addition of 1.0 g activated charcoal. Evoporation was continued until the content of china disk become viscous. Warm distil water was added and filtered through Whatman filter paper No. 1. The filtrate was collected in to 25 ml volumetric flask and to make up it 25 ml. The china disk was washed with little amount of hot water for about 5-6 time. Filtrate was collected in a flask and this hydrolizate was transferred to a 10.0 ml beaker and add 4.0 ml distil water and 2.0 ml of 5 N-NaOH was also added. Further, 0.1 ml sodium nitroproside and 2.0 ml glycine solution (3%) was also added. At last, 4.0 ml metaphosphoric was added to develop color. The intensity of color was measured along with the blank on spectrophotometer at 450 nm.

Homogenized sample (0.2 g) was transferred in 100 ml conical flask by adding 10.0 ml H_2SO_4 (19 N) to determine the tryptophan content (Spice and Chamber, 1949).The content of conical flask was kept for 12.0 hr in dark place. After expiry of period, 1.0 ml distilled water, 1.0 ml p-dimethyl amino benzaldehyde and 0.1 ml of sodium nitrate solution (0.45% in water) were added. The intensity was monitored at 620 nm at spectrophotometer.

The samples were centrifuged at 10,000 rpm at room temperature for 10 minutes to estimate the lysine content (Cancon, 1975). Very fine barley grain sample (50.0 g) was taken and added 50 ml buffer solution (0.5 N tetera sodium pyrophosphate, HCl buffer pH 9.4) then shaken gently and kept at room temperature for 2.0 hr thereafter it was centrifuged at 10,000.0 rpm for 10 minutes. The supernatant was collected and observed the readings at 420 nm.

Total mineral content was estimated by following the method as described by Hart and Fisher (1971). 5.0 g dried sample at 70 °C, was taken and transferred to ash less filter paper. Ignition of sample was carried out on non luminous flame in preweighted silica crucible. The crucible was placed into muffle furnace maintained at 525 to 550°C for about 5-6 hr.

Statistical analysis: Analysis of variance (ANOVA) was performed on each of the data set to confirm the validity of data (Gomez and Gomez, 1984)

Results and Discussion

This may be explained by additional nitrogen in the soil fixed by the legume crop being transferred to the barley roots, although it is possible that other factors that improved barley growth enabled the barley plants to acquire their own N more efficiently. The data of available nitrogen content was found to increase in the soil samples during the year 2006 (131 kg ha^{-1}) in comparison to year

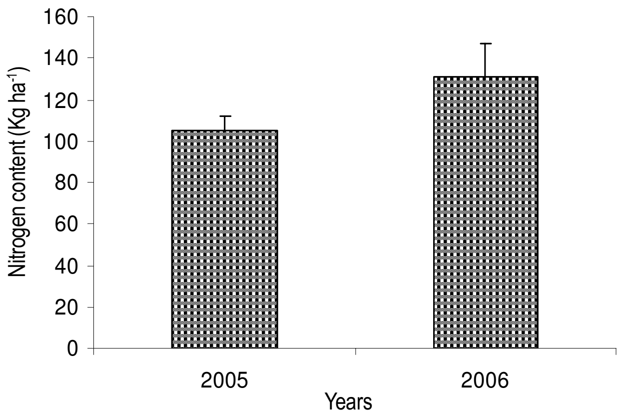

Fig. 1: Available nitrogen content (kg ha^{-1}) in the soil sample of field. All values are mean of three replicate ± S.E. Students t-test (Two tailed) N.S. ($p<0.05$)

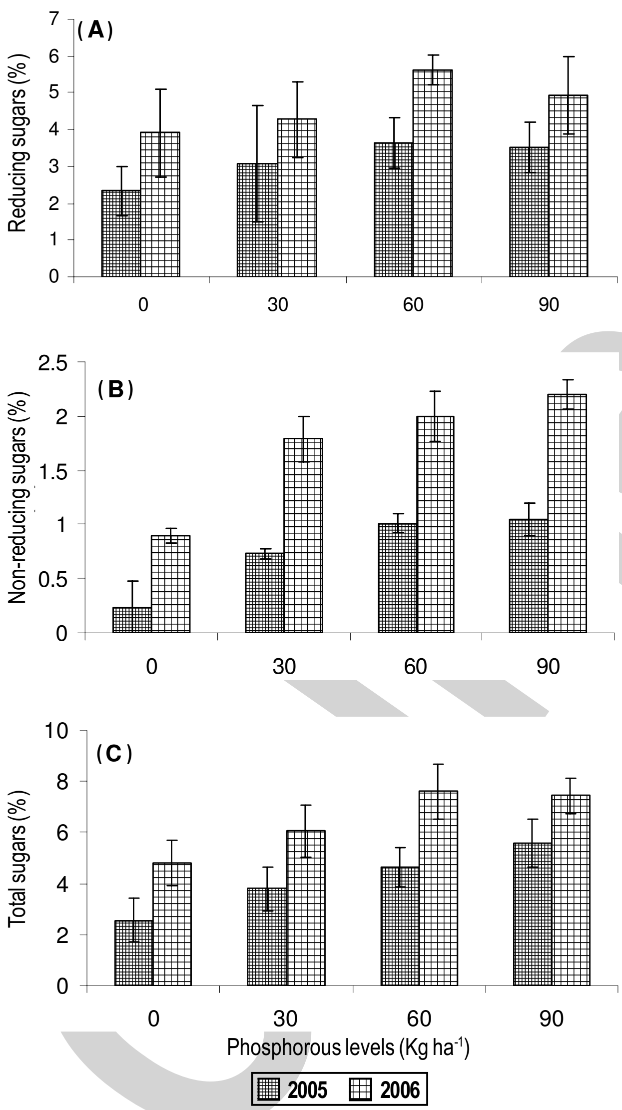

Fig. 2: Effects of phosphorous levels on reducing (A), non-reducing (B) and total (C) sugar contents (%) of *H. vulgare* (NDB-1050). All values are mean of three replicate ± S.E. ANOVA (two way) ($p>0.05$)

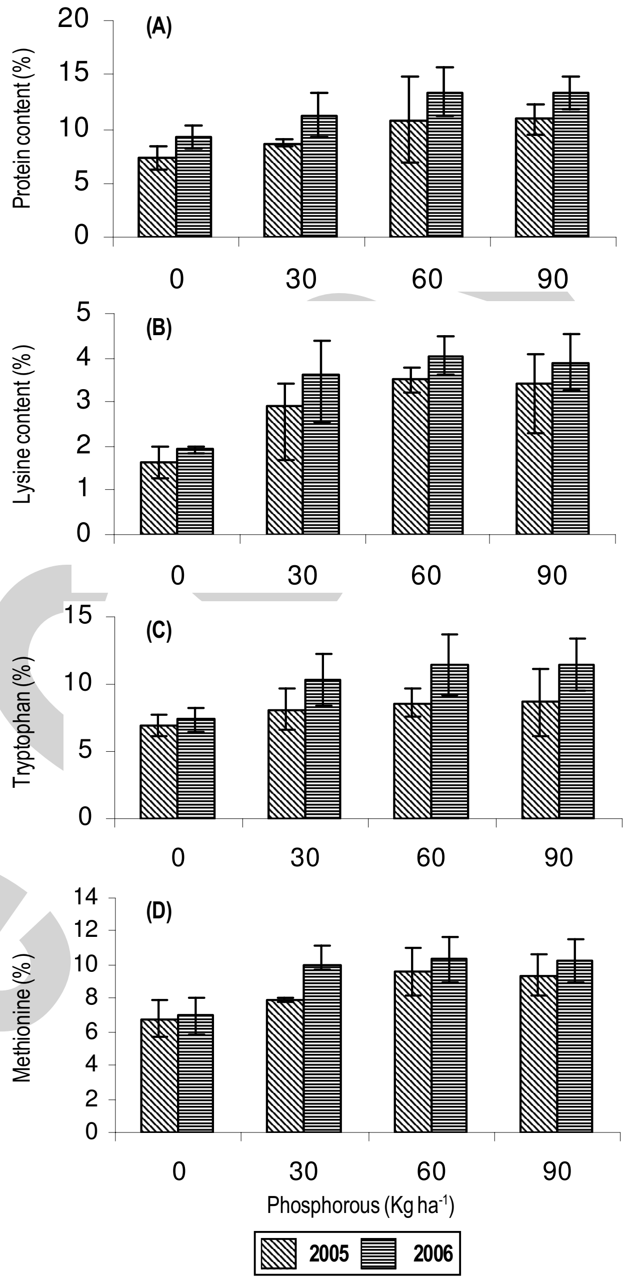

Fig. 3: Impact of phosphorous levels on protein (A), lysine (B), tryptophan (C) and methionine (D) content (%) of *H. vulgare* (NDB-1050). All values are mean of three replicate ± S.E. ANOVA (two way) ($p>0.05$)

2005 (105 kg ha^{-1}). It is also influenced due to mixed cropping system with chickpea because of atmospheric nitrogen fixation process. Results of available nitrogen content were depicted in Fig. 1 as recorded comparatively during the period in the year 2005 and 2006. Increase in the available nitrogen content, was found comparatively non significant ($p<0.05$). Plants have evolved many morphological and enzymatic adaptations to tolerate in low phosphorous concentration. Reducing sugar, non reducing sugar and total sugar content of *H. vulgare* grain was influenced by phosphorous concentrations (Fig. 2). Maximum reducing sugar (3.64%) was estimated at 60 kg P_2O_5 ha^{-1} during the year 2005

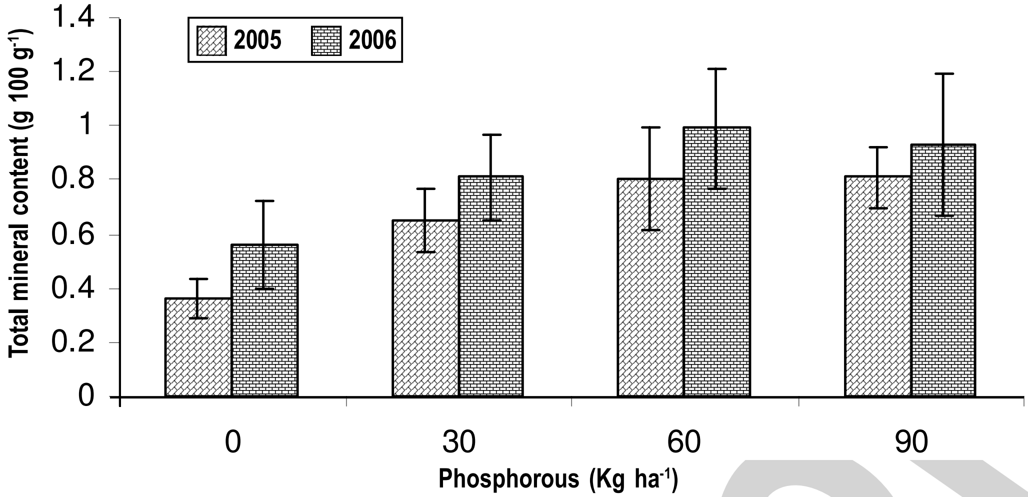

Fig. 4: Effects of phosphorous levels on total mineral content (g 100g^{-1}). All values are mean of three replicate ± S.E. ANOVA (two way) ($p>0.01$)

while maximum non reducing sugar (1.05%) and total sugar (5.57%) were estimated at 90 kg P_2O_5 ha^{-1} during the same year. Minimum reducing sugar (2.33%), non reducing sugar (0.23%) and sugar (2.56%) were found under control conditions. In the next year (2006), reducing sugar (5.62%) was estimated maximum in 60 kg P_2O_5 ha^{-1} and non-reducing sugar (2.0%) was found same in both phosphorous loadings at 60 kg P_2O_5 ha^{-1} and 90 kg P_2O_5 ha^{-1}. Total sugar content (7.74%) was estimated maximum at 90 kg P_2O_5 ha^{-1} during the 2006. Maximum and minimum protein content at 60 kg P_2O_5 ha^{-1} during the year 2006 was found 13.43 and 9.21% respectively. Lysine, tryptophan and methionine contents were estimated maximum 4.04, 11.52 and 10.31%, respectively at same phosphorous level and year (Fig. 3). Minimum lysine, tryptophan and methionine contents were estimated 1.93, 7.38 and 6.96%, respectively under controlled conditions during the year 2006. Maximum total mineral content (Fig. 4) in whole grain of H. vulgare 0.99 g 100 g^{-1} was obtained by the application of 60 kg P_2O_5 ha^{-1}. However, increasing phosphorous levels beyond 60 kg P_2O_5 ha^{-1} could not be able to affect the biochemical parameters of *H. vulgare* grain.

The major management practices employed in mixed cultures to attain good yield includes the enhancement of microclimatic conditions , improved utilization and recycling of soil nutrients, improved soil quality, provision of favorable habitats for plants and stabilization of soil, among others. All the data obtained from study during the year 2005 and 2006; an interesting trend was found in each data sets with a significant improvement in all parameters in comparison to previous year. The growth of nitrogen fixing trees is often limited by the available supply of phosphorous (P) in the soil, and any factor limiting growth may also limit rates of nitrogen fixation (Binkley *et al.*, 2003). It is possible that there was an increase in the soil N made available by the leguminous chickpea species, and this could be another reason why there was an increase in *H. vulgare* shoot mass per plant with intercropping of chickpea. Most annual crop mixtures such as those involving cereals and legumes are grown almost at the same period and develop root systems that explore the same soil zone for resources. This trend represented the importance and benefits of mixed cropping system with chickpea, that plant increased the availability of N through biological processes (Gunes *et al.*, 2007). In most of the plants, inorganic nitrogen is obtained from the soil in the form of nitrate. In contrast to some negative effects on yield, root systems in mixtures may provide some of the major favorable effects on soil and plants. The effect that application of nitrogen-phosphorous (NP) rates exerts on some parameters of nitrogen metabolism and on yield in aubergine plants (*Solanum melongena*). High dry matter from phosphorous fertilization encouraged more mineral accumulation which favored the development of tiller and formation of large number of grains, finally resulted in to higher production (Kumar and Madam, 1995). Increase in phosphorous level increased the quality of carbohydrate that is assimilated by the plants. In legumes, phosphorous deficiency specially affects symbiotic fixation of N_2 by limiting growth and survival of rhizobia, nodule formation and functioning and host plant growth.

Plant growth is dependent on an adequate nitrogen (N) supply in order to form amino acids, proteins, nucleic acids and other cellular constituents for different plant parts and translocated to the developing kernels in the plants. Application of phosphorous produced pronounced effect on test weight. Increase in test weight with increasing levels of phosphorous. Under the high level of phosphorous, plant synthesized more photosynthates and storage organ (Arheimer and Liden, 2000). High rate of phosphorous fertilization encourage vegetative growth and formation of well developed root system which efficiently absorb maximum nutrients uptake from the soil (Sharma and Gupta, 2002). In the case of without application of phosphorous could not well developed root system which not cable to absorb maximum nutrient uptake from the soil (Kumar *et al.*, 1997). In addition, phosphorous deficiency has previously been reported to decrease nodule mass more than host growth in plant (Laxminarayana and Thakur, 1999). Reports on P requirements for nodule formation and functioning are controversial: phosphorous deficiency may increase, decrease or not affect the nodule number per unit of shoot mass (Tang *et al.*, 2001). In case of mixed cropping (cereals + pulses), cereal do not absorb nitrogen from the soil, which is fixed by pulse crop. It is available after mineralization of pulse crops. Plants synthesize more photosynthatases (sugar) because phosphorous is an essential element required for the process of photosynthesis (Tsvetkova *et al.*, 2003). Phosphorous influenced the protein content significantly because it is an essential constituent of majority of enzymes which are of great importance in the transformation of energy required in ATP activation of amino acid for synthesis of protein. Lysine content as a percentage of protein (mg 100 gm^{-1}) showed a decreasing trend with increase in protein percentage of grains because negative correlation exists between protein and lysine as an improvement of protein quality in cereal. This mechanism is due to the disturbance in nitrogen metabolism which results in the synthesis of large number of proteins and amino acid (Lupez-Cantarero *et al.*, 1997). Increasing doses of phosphorous had increased total minerals in the grains of *H. vulgaris* cultivar, as applied phosphorous increased total uptake of minerals *viz.* N, P, K, Ca, Mn and Mg as well as their translocation into seeds. There is evidence that the mineralization of decomposing legume roots in the soil can increase N availability to the associated crop (Evans *et al.*, 2001).

Phosphorous also encourage vegetative growth and formation of well developed root system which efficiently absorb mineral from soil. Thus, it could be concluded that the mineralization of decomposing legume roots in soil can increase N availability to the associated crop and changes in the phosphorous concentration, also affect the biochemical composition of the plant grains. Mixed cropping could overcome potential nutrient deficiencies, particularly in harvested seeds, and the value of such agronomic arrangements could be in addition to relatively more expensive efforts. Increasing mineral nutrient concentration of the seed is a high priority research task and will greatly contribute to alleviation of micronutrient deficiencies in human populations worldwide.

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