Morpho-physiological and oxidative responses of nitrogen and phosphorus deficiency in wheat (*Triticum aestivum* L.)

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ABSTRACT

Wheat is one of the most important cereal crops in India. Nitrogen and phosphorus are the major nutrients, which are deficient in Indian soil; hence the effect of this deficiency on morpho-physiological response and antioxidant metabolism were studied in wheat to improve the knowledge and understanding of adaptive responses of plants. Two wheat genotypes WH1080 and WH1105 were raised hydroponically for 15 and 25 days, using germination paper with four treatments—T1 (0%N+0%P), T2 (0%N+100%P), T3 (100%N+0%P), T4 (100%N+100%P). It was found that root/shoot ratio increased, however total soluble sugar content decreased. An increase of 8.1% was observed in WH1105 as compared to WH1080 in terms of root/shoot ratio. Lipid peroxidation and H$_2$O$_2$ content increased with decreasing nitrogen and phosphorus levels, which led to production of antioxidant enzyme as superoxide dismutase (EC 1.15.1.1) and catalase (EC 1.11.1.6). It was found that oxidative stress was greater under the deficiency of nitrogen as compared to that of phosphorus.

Key words: Lipid peroxidation, Nitrogen, Oxidative stress, Phosphorus, Wheat.

INTRODUCTION

Wheat, with total production exceeding 760.1 million tonnes, is one of the most important cereal crops in the world. It is a staple food for most of the world’s population (FAO, 2017). Wheat is cultivated extensively in northwestern and central zone of India. Nutrient deficiencies are major factors limiting the productivity and geographical distribution of many agricultural crops (Andrews et al., 2010).

Nitrogen (N) and phosphorus (P) are two of the most important plant nutrients affecting growth and development of wheat. N is an essential constituent of proteins, nucleic acids, and secondary metabolites (Ranjan et al., 2016) and the availability of N is a key determinant of wheat yield (Mohan et al., 2015). Most of the Indian soils are deficient in N and this is likely to occur in light-textured sandy soils leached by heavy rainfall or excessive irrigation and in soil having low organic matter. In Indian conditions, plants get only 30-40% of total applied N fertilizer even if it is applied properly (Ladha et al., 2005).

Availability of P present in soil to plants is often limited due to its strong bonding in insoluble forms. P directly controls the responsiveness of photosynthesis to CO$_2$, plays a vital role in the formation of high energy bonds and membrane phospholipids, and is an integral component of several metabolic reactions and signal transduction pathways (Duff et al., 1989). Approximately 43% of cultivated soils worldwide are inadequate in P supply (Zhu et al., 2012). Various studies have been done in tobacco (Rubio-Welhelmi et al., 2011), mulberry (Tewari et al., 2007), rice (Huang et al., 2004) etc., but a comprehensive study involving individual and combined effect of N and P stress has not been reported yet.

Therefore, the present research was conducted to study the morpho-physiological responses and antioxidant metabolism of wheat under nitrogen and phosphorus deficiency.

MATERIALS AND METHODS

Plant material and treatments: Fresh seeds of two wheat genotypes WH1080 and WH1105 were obtained from, Department of Genetics and Plant Breeding, CCSHAU, Hisar India. Surface sterilized seeds were germinated in petri-plates for two to three days. Uniform sized seedlings were planted on germination paper sheets. The germination paper sheet with seedlings planted on it was tightly folded lengthwise into a cylinder and was wrapped in a piece of polyethylene sheet with the help of rubber bands while making sure that lower 2 cm of the germination paper cylinder remain exposed. These cylindrical germination papers were dipped in Hoagland nutrient solution with different N and P concentrations such as T1 (0%N+0%P), T2 (0%N+100%P), T3 (0%P+100%N), T4 (100%N+100%P). In Hoagland solution Ca(NO$_3$)$_2$ and KNO$_3$ were replaced by...
CaCl$_2$ and K$_2$SO$_4$ respectively for creating nitrogen deficiency while phosphorus deficiency was created by using K$_2$CO$_3$ in place of KH$_2$PO$_4$ with half strength to avoid toxicity (Shaw et al., 1985). The beakers containing cylinders were kept under controlled conditions at temperature 25± 2ºC, humidity level 75% and photoperiod of 16 h light (intensity of 2000 lux) and 8 h of darkness. Nutrients solutions were changed twice a week. After 15 and 25 days of sowing, following physiological and biochemical observations were recorded in both root and shoot:

**Morphological observations:** Root/shoot ratio (on the basis of dry wt.) was taken at 15 and 25 days after sowing.

**Biochemical assay:** Total soluble sugar content (TSC) was determined with the method of Yemm and Willis (1954). The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) present in leaf and root tissues. MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction according to the method of Heath and Packer (1968). The H$_2$O$_2$ content was measured by the method of Sinha (1972). The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to Beauchamp and Fridovich (1971). The catalase activity was estimated according to the procedure described by Aebi (1984).

**Statistical analysis:** The research was conducted in 2014-15 using completely randomized design with 4 Replications. Data was analyzed using OPSTAT software.

**RESULTS AND DISCUSSION**

**Root/Shoot ratio:** In the present study, it was found that the root/shoot ratio increased under the N and P deficiency. N and P deficiencies significantly decreased stem and leaf weights but not root weight, which led to a significant increase in the root-to-stem ratio (Zhang et al., 2014). The maximum 42.1% (WH1080) and 50.4% (WH1105) increase was observed 15 days after sowing (DAS), which was further increased to 60.1% (WH1080) and 67.1% (WH1105) 25 DAS (Fig 1). Wang et al. (2003) also reported that there is an increased root/shoot ratio in wheat at low P level. An increase in root to shoot ratio is considered important for tolerance to low nutrient stress in pigeonpea (Fujita et al., 2004). It was also observed that the increase in root/shoot ratio was more in nitrogen starvation than in phosphorus starvation. And, the WH1105 showed more increase in root/shoot ratio than WH1080.

**Total soluble sugar content:** A decline in total soluble sugar (TSS) content was observed under N and P deprivation. The result showed maximum decrease of 20.8% and 22.2% in shoots 15 DAS and 25 DAS respectively (Fig 2A). This may be due to decrease in photosynthetic rate under N deficiency (Sinclair, 2002). Roots also showed a decline which was 27.3% (15 DAS) and 33.3% (25 DAS) in T1 treatment (Fig 2A). Nitrogen deficiency resulted in higher decline of total soluble sugar content than in case of phosphorus deficiency as observed in treatments T2, T3. In conformity with the present results, Khaveri- Nejad et al. (2013) also showed significant decrease in sugar content of roots and shoots of P-deficient tomato plants. In addition to above, Genotype WH1105 showed less decrease in total soluble sugar content than WH1080.

**Lipid peroxidation level:** Lipid peroxidation was measured in terms of malondialdehyde (MDA) content. Increase in MDA content under N and P deficiency (Fig 2B) resulted in increased oxidative stress and impairment in membrane function. As compared to control, 28.2% - 71.8% increase in WH1080 and 27.9% - 66.7% increase in WH1105 was observed 15 DAS in shoots, which further increased to 70.5% to 2.3 fold in WH1080 and 57.7% to 2 fold in WH1105 (Fig 2B) 25 DAS. The MDA content was more in the roots as compared to shoots (Fig 2B). This may be because the roots encounter N and P deficiency earlier than the other parts of the plant. An increase in lipid peroxidation levels under N and P deficiency has been reported earlier in maize (Tewari et al., 2004) and mulberry (Tewari et al., 2007). In roots, a 2.4 fold increase was observed in both WH1080 and WH1105 15 DAS (Fig 2B) while a 2.5 fold increase was observed 25 DAS. An increase in lipid peroxidation results

![Fig 1: Effect of nitrogen and phosphorus deficiency and their combination on root/shoot ratio (on the basis of dry wt.)](image-url)
in membrane injury which leads to senescence (Buchanan-Wollaston et al., 2003) and it may work as a long-term survival strategy (Kandlbinder et al., 2004). It was found that nitrogen deficiency caused more increase in MDA content than phosphorus deficiency. There was no considerable difference between both the genotypes however, WH1105 showed less increase in MDA content than WH1080.

\[ \text{H}_2\text{O}_2 \text{ content:} \] It has been proposed that the generation of reactive oxygen species (ROS) in the early stage of signaling cascade leads to the protection from different abiotic stresses (Corpas et al., 2001). An 61.8% - 79.4% increase in WH1080 and 60.6% - 78.8% increase in WH1105 was observed 15 DAS in shoots, which further increased to 57.1% - 85.7% in WH1080 and 52.9% - 82.4% in WH1105 25 DAS.
The maximum increase was observed in T1 treatment, followed by T2 and T3.

The N and P deficiency resulted in more $\text{H}_2\text{O}_2$ production in roots as compared to shoots. An 75.7% - 97.3% increase in WH1080 and 72.2% - 97.2% increase in WH1105 was observed 15 DAS in roots. Similar trend was observed 25 DAS in roots with 78.9% to 2.1 fold increase in WH1080 and 73.7% - 97.4% increase in WH1105 (Fig 2C). Shin et al. (2005) proposed that ROS are a common element in plant signal transduction cascades in response to nutrient deprivation. N deficiency caused more increase in $\text{H}_2\text{O}_2$ content as in WH1080 it caused 69.7% and 83.8% increase in shoot and root respectively 15 DAS whereas P deficiency caused maximum 61.8% and 75.7% increase in shoot and root respectively (Fig 2C). In the case of nutrient deprivation, increased ROS activity alters the growth pattern of lateral roots in order to facilitate the exploration of new regions of the soil to find additional nutrient elements (Correa-Aragunde et al., 2004).

**Superoxide dismutase activity**: Superoxide dismutase (SOD) is the first enzyme in detoxification process and converts superoxide radicals to $\text{H}_2\text{O}_2$ at a very fast rate (Gratao et al., 2005). In the present study it was found that the superoxide dismutase activity increased under N and P deficiency. It was observed that the SOD content increase up to 2 fold in both WH1080 and WH1105 in shoots 15 DAS. There was no considerable increase 25 DAS in shoots (Fig 3A). Maximum increase was observed in T1 treatment followed by T2 and T3. Hence, N deficiency resulted in higher SOD activity than P deficiency. The maximum 2.1 fold (25 DAS) increase was observed in shoots while 2.2 fold (25 DAS) increase was observed in roots. Although the SOD activity was found higher in roots as compared to shoots region, there was no considerable difference between the two (Fig 3A). Tewari et al. (2007) reported an increase in SOD activity under N and P deficient mulberry plants. Rubio-Wilhelmi et al. (2011) also reported an increase in SOD activity under N deficiency in tobacco plants.

**Catalase activity**: The activity of the $\text{H}_2\text{O}_2$ scavenging enzyme catalase (CAT) also increased under N and P deficiency. In shoots, the maximum 59% and 64.8% increase in catalase activity was observed 15 and 25 DAS respectively while in roots, maximum increase was 80.4% to 93% 15 DAS and 25 DAS respectively. Hence, roots showed more...
increase in catalase activity than shoots (Fig 3B). Tewari et al. (2007) reported an increase in CAT activity in mulberry leaves under N and P deficiency. Kandlbinder et al. (2004) also reported an increase in CAT activity under P deficiency but interestingly a decline in CAT activity was observed under N deficiency in Arabidopsis thaliana. It was found that N deficiency caused maximum 52.5% and 62.2% increase in 15 DAS and 25 DAS respectively in shoots whereas phosphorus deficiency caused 50.1% and 60.4% increase in both 15 and 25 DAS in shoots (Fig 3B). Similar results were found in roots. Genotype WH1105 showed more catalase activity both in shoots and roots. The maximum increase was observed in T1 treatment followed by T2, T3.

CONCLUSION
Finally, these results suggested that N and P deficiency can alter some roots and leaves metabolic characteristics. Root/shoot ratio increased and total soluble sugar (TSS) content decreased under Nand P deficiency in both the genotypes. While both H2O2 content and lipid peroxidation were increased under stress condition, this increase was more in roots than in the shoots.

The antioxidant enzyme system (Superoxide dismutase, Catalase) was induced under stress condition. Also, this effect was more in the roots as compared to the shoots. Maximum increase was observed in T1 followed by T2 and T3. The performance of WH1105 was better in terms of root/shoot ratio, total soluble sugar content and antioxidative enzymes. This study has provided a new insight about the adaptive response of wheat plants under N and P stress which can be further utilised for screening of germplasms for crop improvement. In future, more detailed research can be carried out to understand the molecular and developmental responses of plants under N and P deficiency.

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