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EFFECT OF NITRIC OXIDE SEED PRIMING ON CHILLING INDUCED WATER RELATED PHYSIOLOGICAL ATTRIBUTES IN GERMINATING WHEAT

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ABSTRACT

Seed priming is a pre-sowing treatment used to reduce stress effects in crops. Chilling stress is one of the stresses that affect the most of the important cereal crops. In order to investigate the role of nitric oxide in mitigating chilling induced physiological variations of wheat (*Triticum aestivum* L.) at early seedling development, two experiments were conducted during the year 2012 and 2013 and the data was statistically analyzed in MSTAT-C computer package. Seven wheat genotypes after seed priming in 0, 10^{-4} and 10^{-5} M sodium nitroprusside (as nitric oxide donor) were grown according to completely randomized design with three replicates in optimum growth conditions. Nitric Oxide donor priming at 10^{-4} M concentration was found to be more effective in reducing cold stress. The study of physiological attributes of the seedlings revealed that NARC-2011, Chakwal-50, Punjab-2011 and Faisalabad-2008 are comparatively more tolerant to chilling stress in association with NO priming.

Key words: Chilling, Priming, Nitric Oxide, Wheat

INTRODUCTION

Seed priming is a pre-sowing treatment in which seeds are soaked in water and/or other chemicals for a definite period of time and then dried back before sowing to store moisture contents. Seedling emergence from primed seeds is found to be more vigorous and faster as compared to unprimed seeds. Seeds priming stimulates various biochemical changes in seed which are essential to commence germination process (Ajouri *et al.,* 2004) besides improving its physiology. Seed priming with growth regulators, sugar beet extract, compatible solutes or inorganic salts causes improvement in germination by providing biochemical and physiological adaptations (Pill and Savage, 2008). Wheat, being a staple diet of Pakistani people, occupies a central position in the agriculture. It contributes 2.2 % to GDP and 10.3 % to the value added in the agriculture (PBS, 2014). Various stresses cause decline in productivity of many crops including. Plants acclimatize themselves to stress by some biochemical and physiological modifications. Low temperature stress hinders growth and development of the plants. When chilling-sensitive plants are exposed to Low temperatures they show the symptoms of water-stress/ drought due to reduced leaf turgor and water potential in addition to root hydraulic conductance (Aroca *et al.,* 2003). Stress tolerance in wheat during seedling stage can be increased by hormonal priming before sowing. There has been much research in recent years about various activities of nitric oxide (NO) in mitigating harmful effects of different stresses in plants. During temperature and water stress increase in production of NO has been observed (Leshem, 1996). Data from literature provide evidence that nitric oxide regulates the response of plants towards stress (Hsu and Kao, 2004). In several studies,(Neil *et al*., 2003; Neil, 2007; Neil *et al*., 2008)researchers attempted to discover the role of nitric oxide in mitigating stress and reported provoked tolerance in maize, wheat and tomato by its exogenous application. However, little information is published about cold stress effects and its mitigation by NO in germination, growth and development of cold-resistant cereals. Present study was carried out to analyze the variations in water related physiological aspects in wheat genotypes by chilling, to confirm the effective concentration of nitric oxide in alleviating chilling effects in wheat and to discover chilling tolerant genotypes of wheat.

MATERIALS AND METHODS

Experiments were conducted during the year 2012 and 2013 in the research laboratory, Department of Botany University of Azad Jammu & Kashmir, Muzaffarabad. Seeds of seven Rain fed Wheat (*Triticum aestivum* L.) genotypes, *viz.* Lasani (V1), Faisalabad-2008 (V1), AAS-2011 (V3), Punjab-2011 (V4), Uqab-2002 (V5), Chakwal-50 (V6) and NARC-2011 (V7) were selected to test out their tolerance in cold stress with and/ without priming. Their seeds after priming in different concentration (0, 10^{-4} and 10^{-5} molar) of nitric oxide donor sodium nitroprusside (snp) were grown in Petri dishes lined with double layer of filter paper. Half of the petri dishes containing primed seeds were kept as control and half were subjected to 4 °C for 6 hours according to

Completely Randomized Design (Shibaski *et al*., 2009; Ansari and Sharif-Zadeh, 2012). Seedlings were harvested after 17 days of germination and were used to study water related physiological parameters. Moisture contents and leaf dry matter content were determined by following the method as described by Saura-Mas and Lloret (2007). Relative water content (RWC) was calculated according to Wheatherley (1950). Leaf water loss was calculated using the formula of Xing *et al*. (2004). Transpiration rate (mol/m^2/s) was determined by cobalt chloride paper method with some necessary modifications (Dutta, 2003). Experiments were performed in completely randomized design (CRD) with 3 replicates. Level of Significance for the variations of means was tested using two way ANOVA. Least significant difference (LSD) test at ($\leq$0.05) was used to compare the difference amongst means of the treatments (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Leaf Moisture Content (LMC): Analysis of variance revealed non-significant ($p \geq 0.05$) variations in LMC between two years data. During first study year all the wheat genotypes showed significant variations in LMC. Similar variations were observed during second trial. Growth conditions (GC) and interactions between GC and priming showed non-significant results during both the trials (Fig.1). Maximum bar for LMC was observed from Aas--2011with 10^{-5} M SNP primed samples followed by same M SNP primed Lasani and 10^{-4} M SNP primed Aas-2011 in chilled GC whereas smallest bar for LMC was revealed from 10^{-4} M SNP primed Narc-2011 in unchilled GC. In present project augmentation in leaf moisture content was revealed from wheat varieties by chilling stress and SNP priming compared to control. As SNP priming reduced chilling effect in wheat genotypes by enhancing early emergence and stand establishment that enabled the plants to capture more moisture, nutrients and solar radiation consequently increased LMC was observed. This is consistent to the previous findings of Hosseinzadeh-Mahootchi *et al*. (2013) who reported decrease in LMC by stress and increase by priming.

Relative Water Content (RWC): RWC is the appropriate estimation of water status of the plant in terms of cellular hydration under the probable effect of leaf water potential. Present project revealed that stress can reduce the RWC in wheat varieties. Variations in LMC values from first and second trial were found to be non significantly varied. Interaction of wheat varieties with varied SNP concentrations revealed very highly significantly varied results for RWC from chilled and unchilled conditions during both trials (Fig. 2). As during both the trials maximum RWC was revealed from 10^{-5} M SNP primed Lasani in unchilled GCs significantly followed by 0 M SNP primed samples in same GC. Minimum RWC was revealed by 10^{-4} M SNP primed Narc-2011 significantly varied to RWC of all studied varieties and treatments in both chilled and unchilled growth conditions. However, during second trials Narc-2011 showed intermediate bar for RWC. These results confirmed the findings of previous studies (Halder and Burrage, 2003, Lugojan and Ciulca, 2011; Tan *et al.*, 2008). They reported decrease in RWC in stress conditions compared to control conditions and increase in RWC by SNP priming. In our opinion SNP priming might have helped maintaining RWC by osmotic adjustment and by changing cell wall strectchness of wheat varieties. Maintenance of RWC has been considered to be a stress resistance mechanism. Moreover variations in RWC by different varieties because of varied genetic potential has also been reported by these researchers.

Total Moisture Content (TMC): Total moisture content of plant tissues has been used many times to characterize the internal water stress in plants. Comparison of TMC in varied GC showed that chilling stress cause reduction in TMC in wheat varieties however priming could mitigate its effect (Fig. 3). Variations in TMC from both the trials were found to be varied however non significantly ($p \leq 0.05$) varied. During first trial, maximum TMC was revealed by 0 M SNP primed Narc-2011 in unchilled GC while during second trial Narc-2011 showed maximum TMC with 0 M SNP priming in chilled GC. Minimum TMC was observed from 10^{-4} M SNP primed Lasani in chilled GC during first trial and in second trial minimum TMC was revealed from 10^{-5} M SNP primed Chakwal-50 in chilled GC. Other such interactions showed intermediate TMC significantly varied from maximum and minimum TMC. Ghassemi-Golezani *et al.,* 2012 reported variations in TMC from genotypes due to varied seed vigor. Increase in plant moisture content by priming has been reported by Hosseinzadeh-Mahootchi (2013). Priming reduced chilling effect in wheat genotypes by enhancing early emergence and stand establishment that enabled the plants to capture more moisture, nutrients and solar radiation consequently increased TMC was observed. However they reported increase in TMC in stress conditions. This contradiction in our results might be because of selection of different plant species as well as different stress.

Leaf Dry Matter Content (LDMC): Graph for interaction of wheat varieties with different snp priming and GCs revealed non significantly varied ($p \leq 0.05$) LDMC from same wheat genotype and snp treatment (Fig. 4). However wheat genotypes showed significant difference ($p \leq 0.05$) in LDMC during the second trial. Comparison of two years results showed them non significantly varied. Minimum LDMC was obtained from 10^{-5} M SNP primed Chakwal-50 in unchilled conditions followed by Aas-2011 primed with 10^{-4} M SNP in same GC. During second trial maximum LDMC was revealed by 10^{-4} M SNP primed Narc-2011 in chilled condition followed by 10^{-5} M SNP primed Faisalabad-2008 in same GC. Similar variations in LDMC by stress conditions and priming were reported by Badr-uz-Zaman *et al*. (2010; 2012). They reported decrease in DMC in stress conditions in most of the genotypes. In present project stress mediator was used for priming so it mitigated stress effect in wheat genotypes by enhancing early emergence and stand establishment that enabled the crop to capture more moisture, nutrients and solar radiation and thus increased dry matter production. During seed priming the rapid imbibition occurs which disrupt cell membrane and cause localized cells in cotyledon and embryonic axis of seed (Powell and Matthews, 1978) and is known to provide oxygen and thus the seedling could experience a less systematic resistance, which ultimately would result in more dry matter production compared to unprimed seeds. Inducing a priming technique would modify photosynthetic efficiency, as the varied increment in leaf area, produced significantly increased in dry matter production.

Leaf Water Loss (LWL): In present study significant ($p \leq 0.05$) effect of stress on LWL of studied wheat genotypes and SNP priming was revealed during both the experiments (Fig. 5). Maximum LWL was revealed from 0 M SNP Narc-2011 in chilled growth conditions followed by same WV in unchilled GC. 10^{-5} M SNP primed Chakwal-50 in unchilled GC during both trials. All other interactions revealed intermediate LWL between maximum and minimum LWL of Narc-2011 and Chakwal-50. This is in consistent with Tan *et al.,* 2008 who reported decrease in LWL in wheat varieties by snp treatment. LWL observed from different wheat varieties was significantly varied during both the studied experiments. Such variations in LWL from different varieties were also previously reported by Lugojan and Ciulca. 2011. They suggested that the degree of variations in genotypes was ample to offer several scopes for the selection of traits in order to improve tolerance in wheat genotypes. If water loss is greater than water uptake, air bubbles can form in the xylem. Plants reduce water loss by closing their stomata, developing thick cuticles, or by possessing leaf hairs to increase the boundary layer. Stomata are quick to respond to environmental cues to protect the plant from losing too much water, but still allowing in enough carbon dioxide to drive photosynthesis.

Transpiration Rate (TR): Present project revealed decrease in transpiration rate in chilling compared to normal conditions in almost all the studied genotypes. However, priming mitigated chilling effects up to some extent (Fig. 6). As 10^{-4} M SNP primed Faisalabad-2008 showed maximum TR in unchilled GC during both the trials. Minimum TR was revealed from 0 M SNP primed Punjab-2011 in unchilled GC during first trial. In second trial minimum TR was found from 10^{-5} M SNP primed Lasani in unchilled GC significant to all other interactions. Many previous researchers (Veselova *et al.,* 2003; Mata and Lamattina, 2001) have reported decrease in transpiration during stress compared to normal conditions. Variations in TR by different wheat varieties is because of their varied tolerance to chilling stress due to difference in their genes. Hayatu and Mukhtar (2010) reported variations in water use efficiency by different varieties by different treatments in same growth conditions. Thus, our results suggest that alterations in hormones concentrations might be responsible for the opening and closing of stomata which balanced the water delivery in plant body.

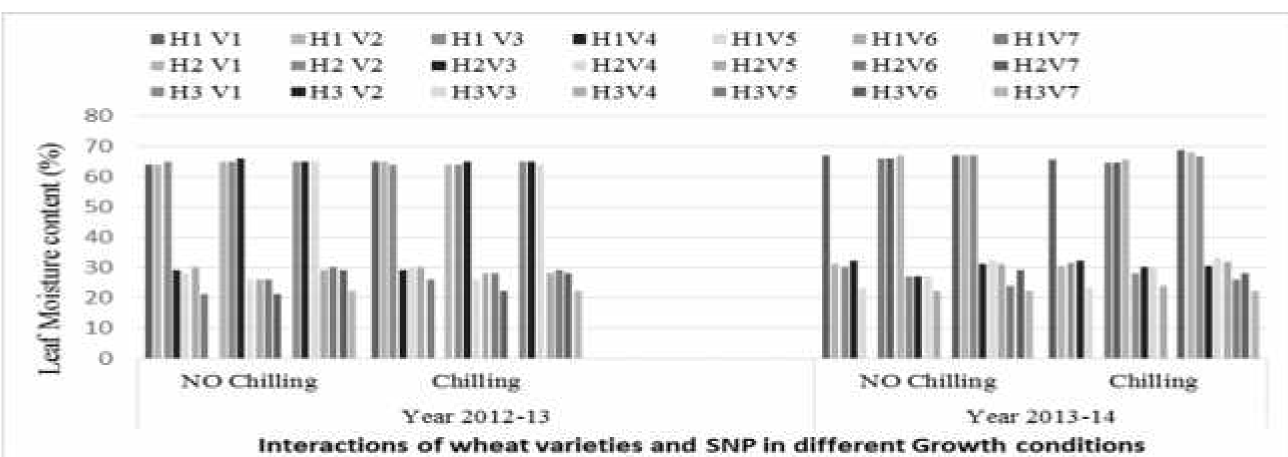

Figure 1. Variation in chilling induced leaf moisture content of wheat genotypes by nitric oxide priming. (H1, H2 and H3 are 0, 10-4 and 10-5 M SNP concentrations respectively), v1, v2. are wheat genotypes as mentioned in material and methods section.

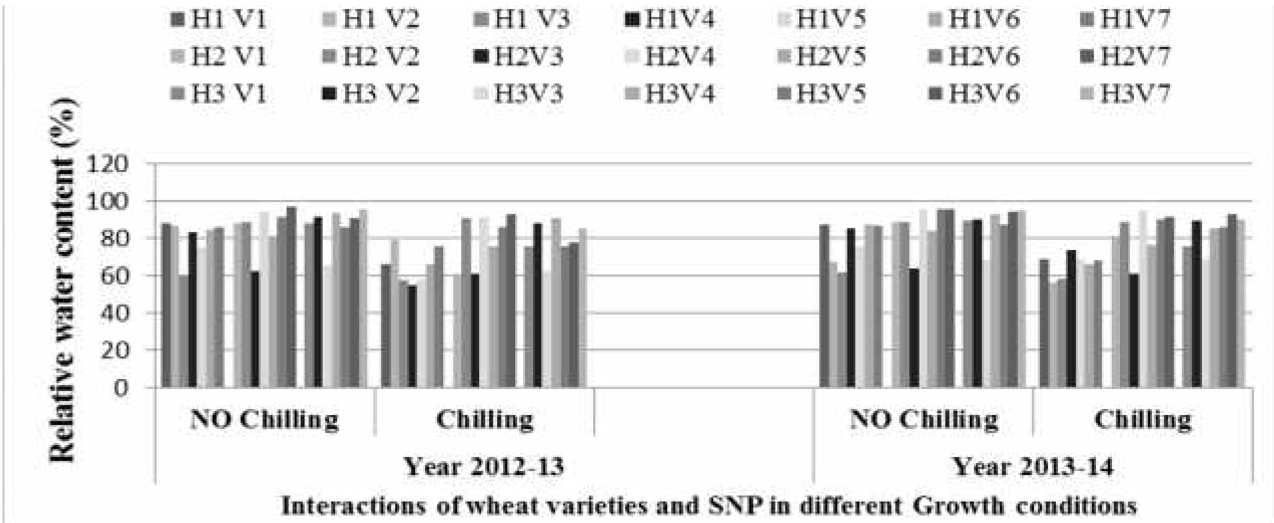

Figure 2. Variation in chilling induced relative water content of wheat genotypes by nitric oxide priming.

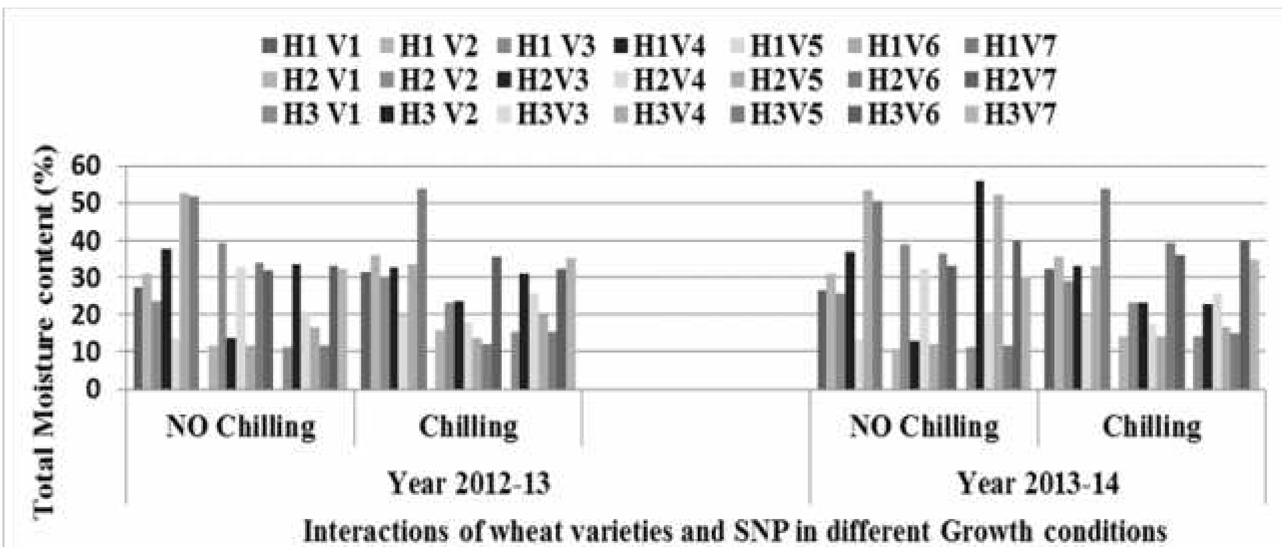

Figure 3. Variation in chilling induced total moisture content of wheat genotypes by nitric oxide priming.

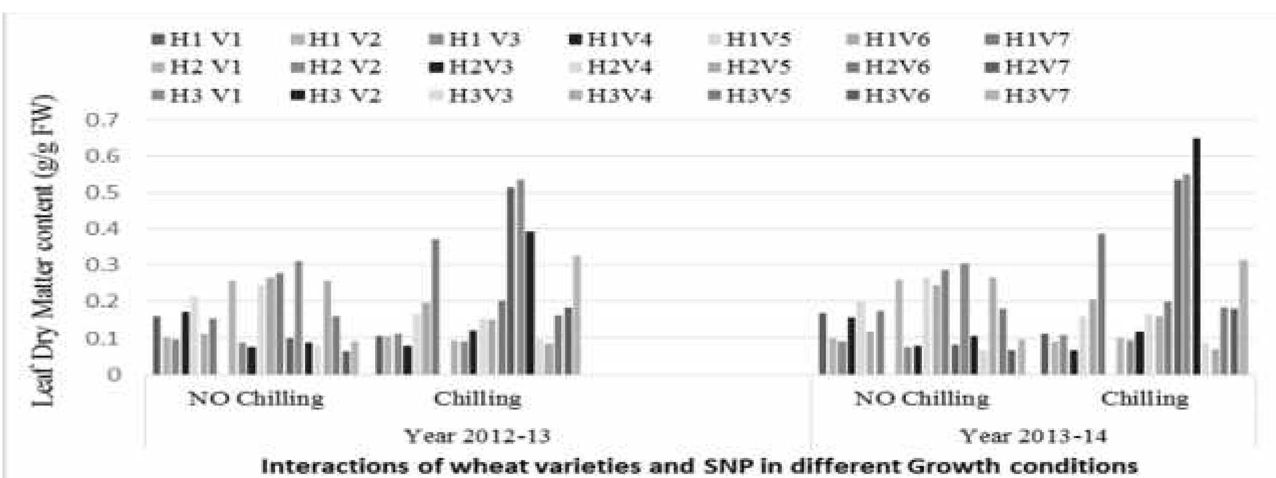

Figure 4. Variation in leaf dry matter content as affected by chilling and snp concentrations.

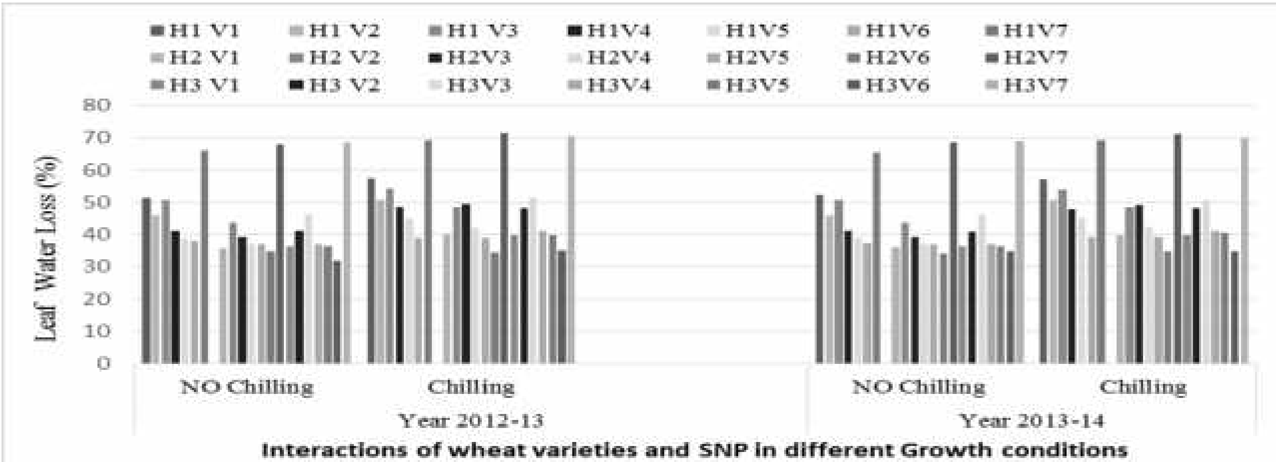

Figure 5: Leaf water loss as affected by chilling and snp.

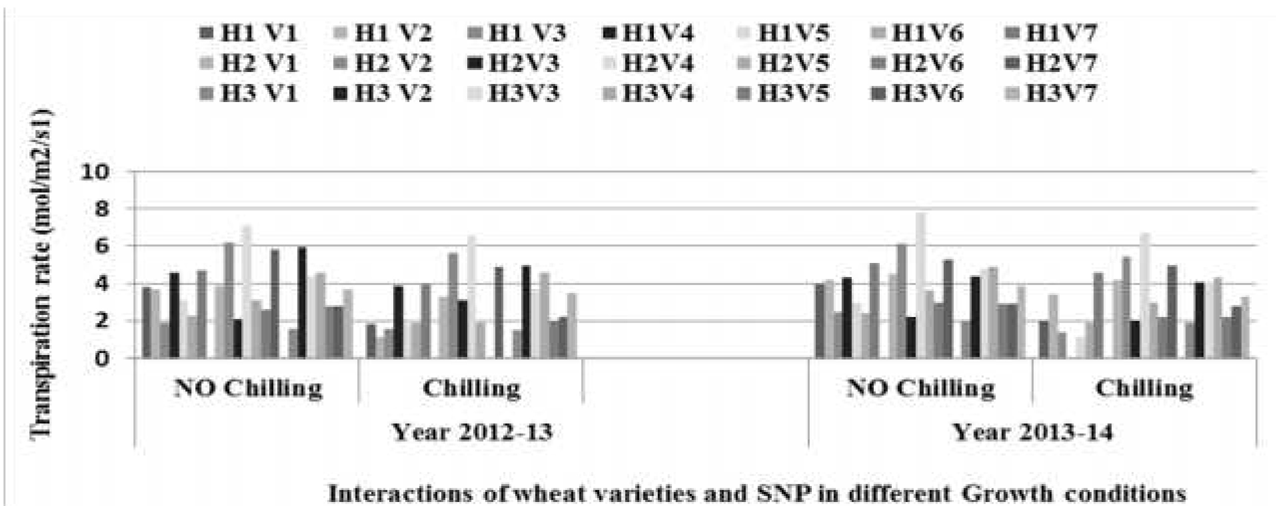

Figure 6: Variation in transpiration rate induced by chilling and snp concentrations.

Conclusion: Water content has substantial importance in plants as it affects their physical characteristics, microbiological stability, sensorial quality and shelf life. In present study chilling stress was found to significantly affect the water related physiology of the wheat. NO donor at 10^{-4} M concentration was observed best in reducing stress effect showing positive results. Moreover, Faisalabad-2008, Narc-2011, Pujab-2011 and Chakwal-50 were found comparatively more tolerant to cold stress among studied wheat genotypes as these genotypes showed promising results for all the studied parameters. It is recommended to apply 10^{-4} M snp to crop plants before sowing in stress hit areas to protect them against stress damage. Additionally, further studies are essential to work out cost benefit ratios of the use of NO as used in this study. This information will definitely be advantageous for stakeholders, especially the farmers.

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