# Greater sensitivity of *Hordeum himalayens* Schult. to increasing temperature causes reduction in its cultivated area

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***Hordeum himalayens* Schult., a lesser-known crop of vital economic importance in central Himalaya, has been showing significant reduction in its cultivated area over the years. The effect of temperature on photosynthetic characteristics of *H. himalayens* during anthesis stage was determined. The data so obtained were compared with another species of *Hordeum* (*H. vulgare* Linn.) to assess their sensitivity to temperature. The data show that photosynthesis in *H. himalayens* is relatively more sensitive to high temperature than in *H. vulgare.* At 30°C, photosynthesis in *H. himalayens* was about 25% less than that of *H. vulgare*. *H. himalayens* also showed a lower stomatal conductance (gs), a lower transpiration rate, a lower water use efficiency (WUE) and lower intercellular $CO_2$ concentration (Ci), but a higher dark respiration rate than *H. vulgare*. It was observed that in *H. himalayens*, the rate of photosynthesis was limited by gs, whereas decreased gs did not lead to corresponding decrease in photosynthesis in *H. vulgare,* probably reflecting differences in the variation in stomatal and non-stomatal control of photosynthesis. In both species, WUE decreased with increase in temperature from 15 to 30°C. The decrease was more rapid in *H. himalayens* than in *H. vulgare*. The data point towards the fact that greater sensitivity of photosynthetic characteristics of *H. himalayens* to high temperature than those of *H. vulgare* could be one of critical factors leading to reduction in its cultivated area. One would also expect that the future environment, which is projected to be warmer and drier, may be still critical to its distribution and productivity. Thus efforts are required for its conservation.**

**Keywords:** *Hordeum himalayens, Hordeum vulgare*, photosynthetic response, temperature, water use efficiency.

THE Indian Himalayan region is a well-known repository of several traditional crops, variously known as lesser-known crops, under-exploited crops or under-utilized crops. These crops have been playing a vital role in the subsistence agricultural systems of the Central Himalaya and hence in the socio-economic development of the region. These crops are considered to be well adapted to harsh environmental conditions, exhibit high degree of resistance to insects, pests and diseases, and have good nutritional and medicinal values[1]. *Hordeum himalayens* Schult., locally known as O-Wa-Jau, is one example of such traditional crops cultivated in Garhwal Himalaya. This crop was earlier grown in large areas (under rainfed conditions), however, in recent times the area sown to it has dropped considerably, and its cultivation today is concentrated in isolated pockets in higher regions of Garhwal Himalaya (> 2000 m asl) as winter crop (rabi crop) either in pure or mixed cropping systems. It is used as human food and cattle feed; its grains are used for making chapattis, sattu and local wine (daru). The causes of reduction in the area of cultivation are poorly understood. While factors like socio-economic and policy factors have been attributed to the overall decline in its cultivated area and thus yield[1], no attempts have been made towards understanding how changing environmental conditions may influence the vital physiological processes that contribute to its growth, productivity and distribution.

There is a growing concern that human-induced climate change would affect the ecophysiological processes of plant species and hence their productivity and distribution[2]. Further, the responsiveness of species to recent and past climate change also suggests that climate change could act as a major cause of extinction of plant species in the near future[3]. The ability of plants to grow and survive in any given environment depends on their photosynthetic capacity[4]. Therefore, studies on gas exchange characteristics may provide valuable information on the functioning of plants in changing environments. Furthermore, photosynthesis has been successfully used as a valuable tool for evaluation of the responses of plants to environmental stresses and for the rapid selection of plants most suitable for different habitats[5,6]. Among the various environmental factors, temperature is the most important one determining the growth and natural distribution of plants by directly or indirectly affecting the photosynthetic processes[7]. An increase in temperature as a result of human-induced global climate change has been predicted[8]. This rise in temperature is likely to have a significant impact on species as well as their habitats, probably by affecting the eco-physiological processes of plant species[2]. It has also been predicted that the future global warming may decrease the yield of several crop species. Therefore, there is a need to assess the responses of plant species to abiotic stresses that are expected to occur as a result of climate change, such as increased temperature. The present investigation deals with photosynthetic response of *H. himalayens* to temperature, and the data so obtained have been compared with another species of *Hordeum* (*H. vulgare* Linn.), which is grown in the plains as well as in the hilly region of Garhwal Himalaya, to gain some insight into the impact of changes in temperature on the performance of these plants.

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Seeds of *H. himalayens* Schult. and *H. vulgare* Linn. were sown during the second week of November in earthen pots (23 cm diameter) containing a mixture of garden soil and farmyard manure. Five seeds of each species were sown in 25 pots each and in order to obtain uniform plants, two plants per pot of each species were maintained with regular watering. Pots were randomly kept in the open in the botanic garden of High Altitude Plant Physiology Research Centre, Srinagar-Garhwal (altitude 550 m amsl; lat. 30°13′; long. 78°48′; average annual temperature 21.3°C; average annual rainfall 711 mm). Photosynthetic measurements were made on flag leaves of four randomly selected plants from different pots for each species under steady state conditions during anthesis stage in the last week of March. For photosynthetic measurements, a climate-controlled, compact minicuvette system (Heinz Walz, Germany) with cuvette temperature ranging from 15 to 30°C (these temperatures approximately corresponded to those experienced by the plant species during their growth period) and a photosynthetic photon flux density (PPFD) of 1600 $\mu$mol $m^{-2} s^{-1}$ (this light intensity was found to be optimum for both species) was used. Recordings were done for a period of 30–60 min for obtaining steady-state conditions. The photosynthetic rate, transpirational water loss, dark respiration rate and stomatal conductance for $CO_2$ (gs) and intercellular $CO_2$ concentration (Ci) were calculated with DIAGAS software according to the equation of VonCaemmever and Farquhar[9]. Instantaneous water use efficiency (WUE) was also computed as a ratio of photosynthetic and transpiration rate. The data were analysed statistically according to Mather[10].

Changes in photosynthetic rate, gs, transpiration rate and WUE of two *Hordeum* species in response to temperature are shown in Figures 1 and 2. It is evident from Figure 1 *a* that there was a significant difference between two species in photosynthetic temperature response, particularly at high temperatures (25 and 30°C). Photosynthesis in *H. himalayens* was slightly higher at low temperatures (15 and 20°C) than in *H. vulgare*, but it decreased significantly at high temperatures; at 30°C, photosynthesis in *H. himalayens* was about 25% less than that of *H. vulgare*. Photosynthesis in plants generally decreases when temperature exceeds optimum values. This indicates the differential sensitivity of photosynthetic activity of the two *Hordeum* species to temperature and a close relationship of photosynthetic activity with the climate of native habitats of these species. The inhibition of photosynthetic rate of *H. himalayens* at high temperatures might contribute to the reduction in its growth, yield and distribution[11].

Lower photosynthesis in *H. himalayens* at higher temperatures may probably be attributed to its reduced gs as overall the gs of *H. himalayens* was approximately 25% lower than that of *H. vulgare* (Figure 1 *b*). Lower transpiration rate and Ci values were also recorded in *H. himalayens* compared to *H. vulgare* (Figure 2 *a* and Table 1). Stomatal limitation has been reported to decrease photosynthesis, Ci and transpiration rate in several plant species[12–14]. Further, in both species gs and Ci values decreased with increase in temperature from 15 to 30°C. The decrease in gs was almost identical in both the species. The Ci values ranged from 267 to 193 $\mu$mol $mol^{-1}$ in *H. himalayens* and 285 to 207 $\mu$mol $mol^{-1}$ in *H. vulgare* (Table 1). Data for photosynthetic rate, gs and Ci further support that in *H. himalayens* rate of photosynthesis was limited by gs, whereas decreased gs did not lead to corresponding decrease in photosynthesis in *H. vulgare*. Further a strong positive correlation between photosynthesis and gs was observed in *H. himalayens*, but correlation was negative in *H. vulgare*, probably reflecting differences in the variation in stomatal and mesophyll control of photosynthesis. Genotypic variations in stomatal and non-stomatal control of photosynthetic rate have been reported[15,16]. According to Jones[15], understanding the relative contribution of different components affecting photosynthesis in

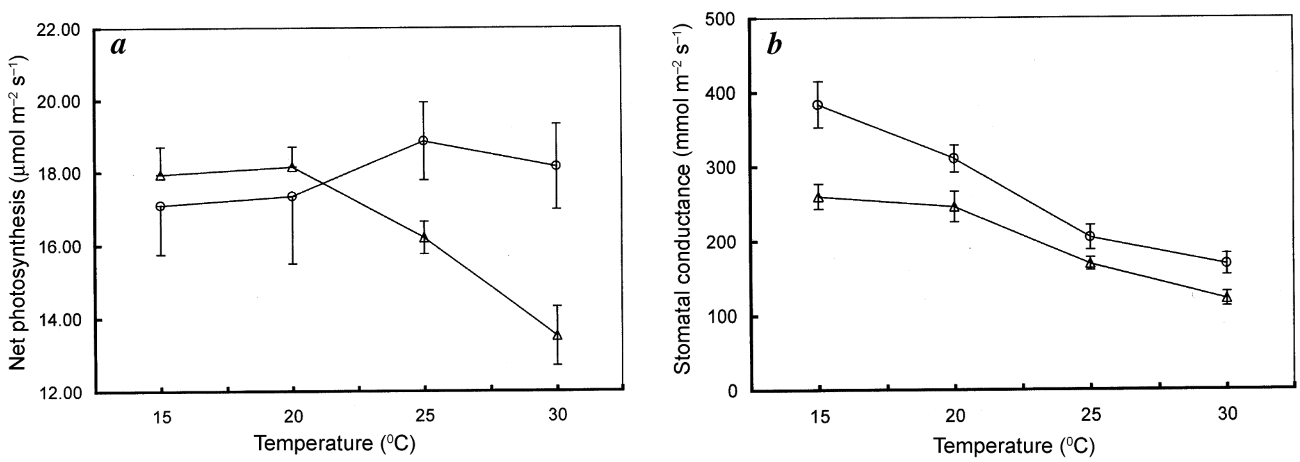


**Figure 1.** Effect of temperature on net photosynthesis (***a***) and on stomatal conductance (***b***) in flag leaves of *Hordeum himalayens* (△) and *Hordeum vulgare* (○). Each value is the mean of four replicates. Vertical bars show standard error.

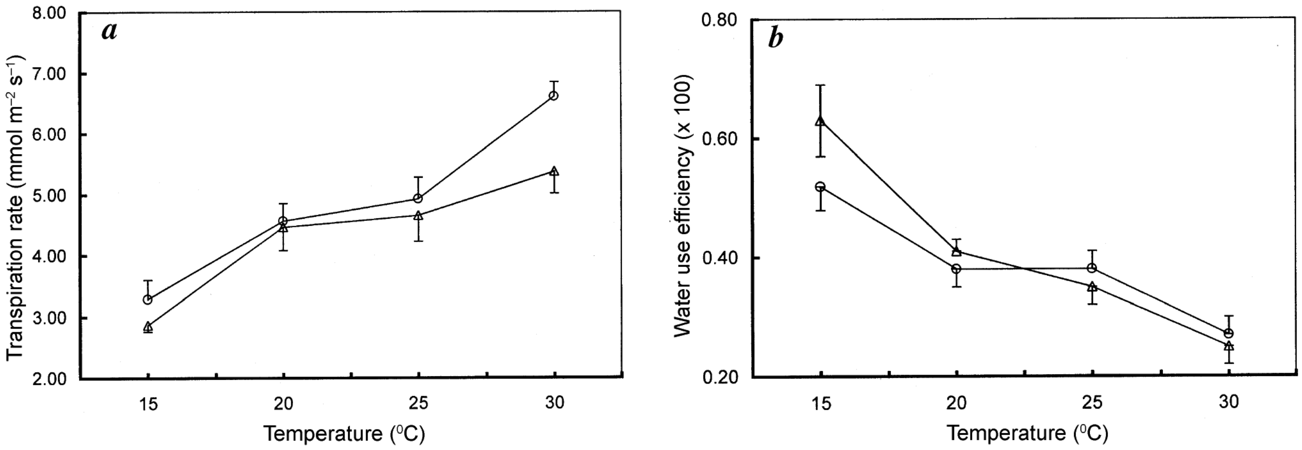


**Figure 2.** Transpiration rate (***a***) and water use efficiency as a function of temperature (***b***) in flag leaves of *H. himalayens* and *H. vulgare.* Symbols and other details as in Figure 1.

**Table 1.** Effect of temperature on Ci values and dark respiration rates in flag leaves of *H. himalayens* and *H. vulgare* ($n = 4 \pm$ SE)

| | Ci (µmol $mol^{-1}$) | | Dark respiration (µmol $m^{-2}$ $s^{-1}$) | |
|---|---|---|---|---|
| Temperature (°C) | *H. himalayens* | *H. vulgare* | *H. himalayens* | *H. vulgare* |
| 15 | 267 ± 23.68 | 285 ± 29.07 | −0.41 ± 0.01 | −0.31 ± 0.04 |
| 20 | 259 ± 26.42 | 277 ± 24.38 | −0.79 ± 0.02 | −0.63 ± 0.04 |
| 25 | 200 ± 12.00 | 235 ± 21.86 | −0.85 ± 0.03 | −0.75 ± 0.06 |
| 30 | 193 ± 13.80 | 207 ± 15.53 | −0.99 ± 0.06 | −0.82 ± 0.11 |

plants is useful for their improvement. However, despite decreases in gs, transpirational water loss increased in both species with increase in temperature. The increase was more pronounced in *H. vulgare.* Transpiration rate is known to depend on stomatal conductance to water vapour and leaf to air vapour pressure difference[17]. In the present study, the latter seemed to be a major factor driving transpiration as leaf to air vapour pressure difference was allowed to increase with increase in temperature in order to simulate field conditions. An increase in transpiration rate and a decrease in stomatal conductance in response to increase in leaf to air vapour pressure difference have been reported in many plant species[5,18].

Further, WUE of *H. himalayens* was higher than that of *H. vulgare* at lower temperatures. With increase in temperature, WUE decreased; the decrease was more rapid in *H. himalayens* than in *H. vulgare* (Figure 2 *b*). Substantial differences in WUE have been reported between species, cultivars or landraces[19]. It has been shown that plants with high rates of photosynthesis and WUE have the potential to grow faster and yield more than species with low photosynthetic rate and WUE under fluctuating environmental conditions[12,20]. However, under limited water availability as in the rainfed areas, WUE is critical relative to photosynthesis for the performance of plants. Therefore, decrease in WUE would lead to a decreased production in *H. himalayens* with increasing temperature[12,13]. There was also a difference in dark respiration between the two species (Table 1); the rate of dark respiration was high in *H. himalayens*, characteristic feature of high altitude plants as they require more respiratory energy to survive and grow under such conditions[21]. An inverse relationship between a plant's yield and its respiration rate has been reported[22]. In both species respiration rate increased with increase in temperature. Increases in rates of respiration in response to high temperatures have been reported for many species[23]. However, increase in respiration rate with increase in temperature was more pronounced in *H. vulgare*.

In summary, the data indicate that photosynthesis in *H. himalayens* is more sensitive to high temperature than *H. vulgare. H. himalayens* also showed a lower gs, lower transpiration rate, lower WUE and lower Ci values, but a higher dark respiration than *H. vulgare*. Exhibition of higher photosynthesis and higher WUE at lower temperatures by *H. himalayens* than that by *H. vulgare,* and vice versa, reflects their adaptation to prevailing habitat temperatures. Results of this study suggest that greater sensitivity of photosynthetic characteristics of *H. himalayens* to high temperature than that of *H. vulgare* could be one of the critical factors leading to reduction in its cultivated area.

One would also expect that the future environment, which is predicted to be warmer and drier, may be still critical to its distribution and productivity. Thus efforts are required for its conservation.


1. Maikhuri, R. K., Nautiyal, M. C. and Khali, M. P., Lesser-known crops of food value in Garhwal Himalaya and a strategy to conserve them. *Plant Genet. Resour. Newsl.*, 1991, **86**, 33–36.
2. Sanz-Elorza, M., Dana, E. D., Gonzalez, A. and Sobrino, E., Changes in the high-mountain vegetation of the central Iberian Peninsula as a probable sign of global warming. *Ann. Bot.*, 2003, **92**, 273–280.
3. Thomas, C. D. *et al.*, Extinction risk from climate change. *Nature*, 2004, **427**, 145–148.
4. Berry, J. A. and Downton, W. J. S., Environmental regulation of photosynthesis. In *Photosynthesis, Vol. II, Development, Carbon Metabolism and Plant Productivity* (ed. Govindjee), Academic Press, New York, 1982, pp. 263–343.
5. Joshi, S. C. and Palni, L. M. S., Clonal variation in temperature response of photosynthesis in tea. *Plant Sci.*, 1998, **137**, 225–232.
6. Isebrands, J. G., Ceulemans, R. and Wiard, B., Genetic variation in photosynthetic traits among *Populus* clones in relation to yield. *Plant Physiol. Biochem.*, 1988, **26**, 427–437.
7. Berry, J. and Bjorkman, O., Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol.*, 1980, **31**, 491–543.
8. Schneider, S. H., The global warming debate heats up: An analysis and perspective. *Bull. Am. Meteorol. Soc.*, 1990, **71**, 1292–1304.
9. VonCaemmever, S. and Farquhar, G. D., Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, 1981, **153**, 376–387.
10. Mather, K., *Statistical Analysis in Biology*, Chapman and Hall, London, 1973.
11. Fisher, R. A., Bidinger, F., Syme, J. R. and Wall, P. C., Leaf photosynthesis, leaf permeability, crop growth and yield of short spring wheat genotypes under irrigation. *Crop Sci.*, 1981, **21**, 367–373.
12. Jones, H. G., *Plants and Microclimate: Quantitative Approach to Environmental Plant Physiology*, Cambridge University Press, Cambridge, 1992.
13. Lawlor, D. W., Photosynthesis, productivity and environment. *J. Exp. Bot.*, 1995, **46**, 1449–1461.
14. Rodriguez, Sanchez, Perez, P. and Martinez-Carrasco, R., Photosynthesis, carbohydrate levels and chlorophyll fluorescence estimated intercellular $CO_2$ in water stressed *Casuarina aquisetifolia* Forst and Forst. *Plant Cell Environ.*, 1999, **22**, 867–873.
15. Jones, H. G., Partitioning, stomatal and non-stomatal limitations to photosynthesis. *Plant Cell Environ.*, 1985, **8**, 95–104.
16. Ni, B.-R. and Pallardy, S. G., Stomatal and non-stomatal limitations to net photosynthesis in seedlings of woody angiosperms. *Plant Physiol.*, 1992, **99**, 1502–1508.
17. Alexander, J. D., Donnelly, J. R. and Shane, J. B., Photosynthetic and transpiration responses of red spruce understorey trees to light and temperature. *Tree Physiol.*, 1995, **15**, 393–398.
18. Rawson, H. M., Begg, J. R. and Woodward, R. G., The effect of atmospheric humidity on photosynthesis, transpiration and water use efficiency of leaves of several plant species. *Planta*, 1977, **134**, 5–10.
19. Cabuslay, G. S., Ito, O. and Alejar, A. A., Physiological evaluation of responses of rice (*Oryza sativa* L.) to water deficit. *Plant Sci.*, 2002, **163**, 815–827.
20. Zhang, J. W., Marshall, J. D. and Fins, L., Correlated population differences in dry matter accumulation, allocation and water use efficiency in three sympatric conifer species. *For. Sci.*, 1996, **42**, 242–249.
21. Miroslavov, E. A. and Kravkina, I. M., Comparative analysis of chloroplasts and mitochondria in leaf chlorenchyma from mountain plants grown at different altitudes. *Ann. Bot.*, 1991, **68**, 195–200.
22. Palit, P., Kundu, A., Mandan, R. K. and Sircan, S. M., Productivity of rice plant in relation to photosynthesis, photorespiration and translocation. *Indian J. Plant Physiol.*, 1979, **22**, 66–74.
23. Pearcy, R. W., Acclimation of photosynthetic and respiratory carbon dioxide exchange to growth temperature in *Atriplex lentiformis* (Torr.) Wats. *Plant Physiol.*, **59**, 795–799.


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# A computer-based identification of variable number tandem repeats in White spot syndrome virus genomes

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**Three complete genome sequences of White spot syndrome virus (WSSV) from Thailand, Taiwan and China, were analysed for the presence of tandem repeats. Thirteen microsatellite and three minisatellite loci which could serve as potential molecular markers for studying and understanding the genetic and epidemiological relationship among WSSV strains were identified. Additionally, we report the presence of two new polymorphic megasatellite loci within the genomes.**

**Keywords:** Microsatellites, minisatellites, megasatellites, variable number tandem repeats, White spot syndrome virus.

WHITE spot syndrome virus (WSSV), an important shrimp virus and one of the largest animal viruses is associated with shrimp mortalities, causing huge economic losses to cultured shrimp worldwide. WSSV has a large genome size; its size has been reported as 292,292 base pairs (bp)[1], 305,107 bp[2] and 307,287 bp[3] for Thailand, Taiwan and China isolates respectively. Since these three isolates show little variation across their genomes with much of their predicted proteins showing no sequence homology to any other viral genes or genes from other organisms[1,2,4,5], knowledge regarding the evolution, epidemiology or the pathogenic mechanism of this organism has been limited.

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