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The effects of rising temperature on cell viability, relative water content and proline accumulation of Plants

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ABSTRACT

Gases emissions due to human activities are substantially adding to the existing concentration of greenhouse gases, particularly carbon dioxide, methane, CFC at nitrous oxides. Different global circulation models predict that greenhouse gases will gradually increase worlds average ambient temperature. According to a report of the intergovernmental panel on climate change (IPCC), global mean temperature will rise 0. 3% °C per decade reaching to approximately 1 and 3% °C above the present value by years 2025 and 2100, respectively and leading to global warming. Heat stress due to high ambient temperature is a serious threat to crop production worldwide. So, keeping the above in view a research program was planned, and an attempt was made to study the eco-physiological parameters and how these contribute in mitigating the adverse effect of heat stress in potato crop. For this, potato cultivars of diverse nature, namely heat susceptible Kufri Ashoka andheat tolerant Kufri Surya were evaluated for eco- physiological attributes namely cell viability, Relative water content, and Proline content. The experiments were conducted in plastic pots which were exposed at predetermined range of temperature that is 20, 30 and 40. Sampling was done at 45, 60 and 75 days after planting (DAP) and one plant/pot was used for taking observations. Significant differences were noticed in tolerant and susceptible cultivars. It was noticed that Kufri Surya maintained a higher Proline and relative water content at all growth stages under all temperature regimes included in the present study. In Kufri Ashoka the metabolic injury was almost double the injury experienced by Kufri Surya. The results in cell viability test indicated that genotypic differences exist in Kufri Ashoka and Kufri Surya. In overall performance, the higher relative water content, cell viability and higher leaf proline in Kufri Surya suggest that through these traits individually as well as in coordination this Genotype could sustain its functional integrity and deviated least during heat stress. Therefore selection of potato cultivars early in clonal generations, on the basis of these criteria/traits may expedite and help to develop more heat tolerant potato cultivars and thereby can equip potato production system better for the projected climate change inevitable due to global warming.

Key words: *Global Warming, Heat stress, Clonal generations*

Introduction

Heat stress due to increased temperature is an agricultural problem in many areas in the world (Hall, 2001). Transitory or constantly high temperature cause an array of morpho-anatomical,physiological and biochemical changes in plants, which effect plant growth and development and may lead to drastic reduction in economic yield (Mirza *et al.*, 2013). The current global scenario of threatened food security due to climate change and decrease in cultivable land under food crop requires growing of potatoes under abiotic stresses, particularly of heat, in conventional regions and in non -traditional areas of tropical zones. Heat stress effects plant growth throughout its ontogeny, though heat threshold level varies considerably at different developmental stages (Wahid and Close, 2007). For instance, during seed germination, high temperature may slow down or totally inhibit seed germination, depending on plant species and intensity of stress. At later stages, High temperature may adversely affect photosynthesis, respiration, water relations and membrane stability (Niu and Xiang, 2018; Sonal *et al.,* 2014; Hassan *et al.,* 2020).

Potatoes actually have a fairly low optimum growth temperature, and it is likely that they experience some heat stress even during season when temperatures are more normal (Rykaczewska, 2015). For vine growth, the optimum temperature is around 23, which for tuber growth the optimum is closer to 18. This disparity in conditions favoringvine versus tuber growth results in one of the common situations associated with heat stress, an imbalance between the above ground and belowground portions ofplant. Therefore, if heat stress is present for a long period around the time of tuber initiation, the result is a situation where the vines appear very big and healthy, but there are very few tubers underneath. Not only is the average daily temperature important, but nighttime temperature is especially critical in determining the timing of tuber initiation and the rate of tuber growth (Rykaczewska, 2015). The optimum day/night temperature for potato growth is generally considered to be 23/ 12. However, at any given day temperature there is a corresponding optimum night temperature. As the day temperature increases a large difference between day and night temperatures is required for optimum growth. The adverse effect of heat stress can be mitigated by developing crop plants with improved thermo-tolerance using various genetic approaches (Janni, 2020). For this purpose, however, a thorough understanding of physiological response of plants to high temperature, mechanism of heat tolerance and possible strategies for improving crop thermo-tolerance is imperative. That is why this study focuses on heat tolerant potato cultivar Kufri Surya and its probable physiological attributes associated with heat tolerance.

Materials and Methods

Pots were kept at optimum moisture level and maintained under natural conditions. The maximum and minimum temperature during planting time in September ranged from 31 - 35°C and 22 to 25 °C respectively. Temperature above 28 °C severely affected the plant growth in potato crop and a temperature of about 70 °C is optimum for good yield. The potato cultivars Kufri Ashoka and Kufri Surya grown in pots were thus exposed to natural heat stress condition of early season (September planted). Kufri Ashoka is an early maturing (70 to 80 days) cultivar and its tubers are large , oval-long, skin white smooth, fleet eyes, and white flesh with medium dormancy (6-8 weeks).The Kufri Surya is a cross between LPT-$_1$(male) heat tolerant line and cultivar Kufri Lauvker (female parent), a popular cultivar of plateau region. The plant is medium tall, erect, vigorous having three to five shoots and dark green open large leaves. The tuber varies from medium to large having oblong shape and white skin with pale yellow color flesh. This is an early maturing cultivar which takes about 75 to 90 days, though it can also be harvested early at 60 days. To study the eco-physiological attributes, the potted plantswere subjected to a range of temperature treatments. In each treatment for pots were marked for recording observation on eco-physiological traits at 45, 60 and 75 days after planting (DAP) in each cultivar. The potted plants were kept at 30 and 40 for two hours in BOD while control plants were kept at room temperature (20). In this way, there were three treatments for different temperature regimes comprising as follows: T1= 20 Room Temperature (control), T2 = 30 and T3 = 40. The cell viability test was done according to the method of Senthil *et al.,* 2002. Relative water content estimates the current water content of the sampled leaf tissue relative to the maximum water content it can hold at full turgidity. It was determined in potato leaves by method of Barr and Weatherley (1962) and the proline content of the leaf was estimated according to the method of Bates *et al*., (1973). Data recorded during the course of study were subjected to statistical analysis by applying the technique of analysis of variance (ANOVA) prescribed for the completely randomized design for pot experiments by Gomez, 1984 .

Results and Discussion

The Results presented in Table 1 shows that Kufri Surya maintains higher relative water content during all the three growth stages (84.9, 89.0 and 85.9% at 45, 60 and 75 DAP, respectively) as against Kufri Ashoka 79.0 , 83.1 and 79.0, respectively). Relative water content increased from 45 to 60 DAP and then declined at 75 DAP. The trend was same the next year in both the cultivars. A declining trend in relative water content (RWC) was observed with increase in temperature, highest RWC being recorded at room temperature (approx. 20 °C). Plants at 40°C experienced about 19- 23% decrease in RWC with respect to plants at room temperature. Data showed that cultivar Kufri Surya showed maximum RWC at room temperature (20°C) on 60 DAPin both the years (96.4 and 96.3%) in comparison to Kufri Ashoka with value of 90.2 and 90.1% during respective years. Osmotic adjustment is an important mechanism of plant tolerance to drought and heat stress (Smith *et al.,* 1989; Ludlow *et al*., 1990). The higher proline content in Kufri Surya seems to work as osmoprotectant and able to maintain higher RWC. Moreover, the plasma membrane is also protected from desiccation induced damaged by the presence of membrane compatible solutes such as higher proline in heat tolerant cultivars. Therefore, a link may exist between capacity for osmotic adjustment and the degree of membrane protection from the effect of heat stress. Similar possibility has been reported earlier in case of dehydration (Janska *et al.,* 1996).

The cell viability test is an important indicator for general metabolic activity and cellular plant functions. Metabolic injury was assessed by triphenyl tetrazolium test.Metabolic injury was less in (Table 2) Kufri Surya during all the three growth stages as compared to Kufri Ashoka (31, 35 and 39). In Kufri Ashoka the injury was almost double the injury experienced by Kufri Surya. Similar trend was followed in the next year. Results suggest that cells of Kufri Surya were more viable and thus experienced less metabolic injury during all the three growth stages and under all the three treatments. Temperature treatments of 20 °C and 30 caused less metabolic injury than temperature stress of 40 which caused about 53% injury at 75 DAP. Cell viability generally decreased with advancing crop age leading to increased metabolic injury.Therefore, the plants at 75 DAP were more susceptible to metabolic injury. The results in the cell viability test indicated that genotypic differences exist in potato cul-

Table 1. Effect of heat stress on relative water content (%) at different growth stages in cultivar Kufri Ashoka and Kufri Surya grown in pots exposed to temperature treatments of 20 (room temperature) 30 and 40 °C.

Treatments *	Ist Year			2nd Year		
	Days after planting					
	45	60	75	45	60	75
Kufri Ashoka						
T1	86.9	90.2	87.0	86.6	90.2	87.3
T2	82.1	87.0	83.2	82.3	87.6	82.5
T3	68.2	72.0	66.6	67.9	71.9	65.6
Mean	79.0	83.1	79.0	78.9	83.2	78.5
Kufri Surya						
T1	93.3	96.4	94.7	92.8	96.3	94.2
T2	92.1	94.2	92.4	92.5	93.6	92.1
T3	69.8	76.5	70.7	70.3	75.1	72.0
Mean	84.9	89.0	85.9	85.2	88.4	it is 6.1
Mean value of Treatments						
T1	90.1	93.3	90.9	89.7	93.2	90.8
T2	86.8	90.6	87.8	87.4	90.6	87.3
T3	69.0	74.3	68.6	69.1	73.5	68.8
CD at 5%						
Cultivar ©	2.9	1.5	3.1	0.7	1.8	2.3
Treatment (T)	3.5	1.8	3.9	0.9	2.2	2.8
C X T	N.S.	N.S.	N.S.	1.2	N.S.	N.S.

*=T1=20 °C (room temp.), T2 = 30 & T3 = 40 °C

Table 2. Effect of heat stress on cell viability (% Metabolic injury as assessed by triphenyl tetrazolium test) at different growth stages in cultivar Kufri Ashoka and Kufri Surya grown in pots exposed to temperature treatments of 20 (room temperature) 30 and 40 ºC during mid-September to December

Treatments *	Ist Year			2nd Year		
	Days after planting					
	45	60	75	45	60	75
			Kufri Ashoka			
T1	21.2	25.0	28.9	22.0	27.2	30.6
T2	25.0	27.2	29.9	26.2	28.1	30.4
T3	49.8	51.6	56.8	51.3	52.3	57.8
Mean	31.1	34.6	38.5	33.2	35.8	39.6
			Kufri Surya			
T1	18.7	22.2	26.3	20.8	24.0	27.8
T2	21.2	25.2	28.3	22.0	26.1	27.8
T3	39.1	44.0	48.4	40.1	45.0	51.7
Mean	26.3	30.5	34.3	27.7	31.7	35.8
			Mean value of Treatments			
T1	20.0	23.6	27.6	21.4	25.6	29.2
T2	23.1	26.2	29.1	24.1	27.1	29.1
T3	44.4	47.8	52.6	45.7	48.6	54.7
CD at 5%						
Cultivar ©	0.6	0.5	0.5	0.3	0.4	0.4
Treatment (T)	0.7	0.6	0.6	0.4	0.5	0.5
C X T	1.0	0.9	0.9	0.6	0.7	0.7

*=T1=20 ºC (room temp.), T2 = 30 & T3 = 40 ºC

Table 3. Effect of heat stress on Proline content (mg/100g FW) at different growth stages in cultivar Kufri Ashoka and Kufri Surya grown in pots exposed to temperature treatments of 20 (room temperature) 30 and 40 during mid-September to December.

Treatments *	Ist Year			2nd Year		
	Days after planting					
	45	60	75	45	60	75
			Kufri Ashoka			
T1	35.4	26.9	16.8	34.4	28.1	17.4
T2	36.4	29.2	18.6	36.2	28.4	19.0
T3	51.9	48.0	23.9	52.2	48.1	24.0
Mean	41.2	34.7	19.8	41.0	34.9	20.1
			Kufri Surya			
T1	37.9	30.1	18.9	38.0	30.7	19.4
T2	38.1	30.9	24.9	38.3	30.2	24.5
T3	56.7	54.0	29.8	57.4	53.9	30.0
Mean	44.2	38.3	24.5	44.6	38.3	24.7
			Mean value of Treatments			
T1	36.7	28.5	17.9	36.2	29.4	18.4
T2	37.2	30.1	21.7	37.3	29.4	21.8
T3	54.3	51.0	26.8	54.8	51.0	27.0
CD at 5%						
Cultivar ©	2.5	N.S.	2.6	0.9	1.2	1.1
Treatment (T)	3 .0	5.7	3.2	1.1	1.5	1.3
C X T	N.S.	N.S.	N.S.	1.6	2.2	1.9

*=T1=20 ºC (room temp.), T2 = 30 & T3 = 40 ºC

tivars used in this study. Their ability to exhibit athermal tolerance mechanism operated at cellular level.

The Results in Table 3 showed that content of proline in leaf tissues was maximum in Kufri Surya (44.2, 38.3 and 24.5 mg/100 g FW at 45, 60 and 75 DAP) in comparison to Kufri Ashoka (41.2, 34.7 and 19.8 mg/100g FW, respectively). Content of proline gradually declined with the age of crop. Mean values of treatment indicate that with increase in temperature treatments, proline content increased highest being recorded at 40 °C and lowest at 20 (room temperature). Both the cultivars showed same trend in both the years with respect to temperature treatments. In general, Kufri Surya showed higher content of proline at all growth stages under all temperature regimes. In many plants, free proline accumulates in response to the imposition of a wide range of biotic and abiotic stresses. Dar *et al.*, (2016) found that the stress resistant varieties accumulated higher levels of free proline then susceptible ones. Proline works as a source of energy, carbon and nitrogen and also protects several enzymes against theinactivating effects of heat during stress (Paleg *et al.*, 1981).

Thus in overall performance, the higher cell membrane stability, cell viability , higher proline and better RWC in Kufri surya suggests that this cultivar could sustain its functional activity and deviated least in eco-physiological traits during heat stress Therefore selection of potato cultivars early in clonal generations, on the basis of these criteria / traits may expedite and help to develop more heat tolerant potato cultivars and thereby can equip potato production system better for the unavoidable climate changes due to global warming.

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