Journal of AgriSearch **5**(3):184-189
An Open Access International Peer Reviewed Quarterly

ISSN : 2348-8808 (Print), 2348-8867 (Online)
https://doi.org/10.21921/jas.5.3.7

Morphological, Physiological and Biochemical Responses of Poplar Plants to Drought Stress

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ARTICLE INFO

Received on	:	17-11-2017
Accepted on	:	27.08.2018
Published online	:	05.09.2018

ABSTRACT

Poplar clone Kranti was selected to assess the morphological, physiological and biochemical responses under drought at different levels of water stress, as it is a common clone used to be grown in Uttarakhand for making paper and plywood. The cuttings of *Populus deltoides* L. (clone Kranti) were exposed to four different watering regimes (100, 75, 50 and 25% of the field capacity) and changes in physiological and biochemical parameters related with drought tolerance were recorded. Alterations in physiological (i.e. decrease in relative water content) and biochemical parameters (i.e. increase in proline and soluble sugar content and build-up of malondialdehyde by-products) occurred in all the three levels of water stress, although drought represented the major determinant. Drought treatments (75%, 50% and 25% FC) decreased plant height, radial stem diameter, harvest index, total biomass content and RWC in all the three watering regimes compared to control (100% FC). Biochemical parameters like proline, soluble sugar and MDA content increased with severity and duration of stress, which helped plants to survive under severe stress. It was analyzed that for better wood yield poplar seedlings should avail either optimum amount of water (amount nearly equal to field capacity of soil) or maximum withdrawal up to 75% of field capacity up to seedling establishment period (60 days). Furthermore, this study manifested that acclimation to drought stress is related with the rapidity, severity, and duration of the drought event of the poplar species.

Keywords: drought, field capacity, Poplar, Proline content, MDA, RWC

INTRODUCTION

Woody crops such as poplars (*Populus*) can contribute to meet the increasing energy demand of a growing human population and can, therefore, enhance the security of energy supply as well as raw material for paper, plywood and matchstick industries. Using energy from biomass increases ecological sustain ability as biomass is considered to play a pivotal role in abating climate change (Hennig *et al*., 2015). Since areas for establishing poplar plantations are often confined to marginal sites, drought tolerance is one of the important traits for poplar genotypes cultivated in short rotation coppice (Hennig *et al*., 2015).

Water stress is an important environmental factor that affects photosynthesis, affecting plant growth and biomass (Akcay *et al*., 2010 ; Kumari, 2015 and Kumari *et al*., 2017). The osmotic adjustment has also been considered as one of the crucial processes in plant adaptation to drought stress. It involves the synthesis and accumulation of small compatible solutes (osmolytes), such as proline, glycine betaine, sugars and some inorganic ions (Chaves *et al*., 2003; Kumari and Sairam, 2013). These compounds help the cells to maintain their dehydrated state and the structural integrity of the membranes so as to provide resistance against drought and cellular dehydration (Ramanjulu and Bartels 2002, Kumari and Sairam, 2013). The level of lipid per oxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid per oxidation.

The high wood production of poplars is strictly linked to soil water availability, which is normally assured by irrigation. Increasing irrigation costs and water shortages worldwide have led to the development of irrigation methods and optimization of level of drought tolerance at different field capacity that minimize water use (Jones, 2004; Kumari, 2015) and to the definition of integrative criteria for assessing drought tolerance in poplar (Marron *et al*., 2003).

(*Populus* spp.) are a diverse and widely distributed genus, which have been commonly studied as a model organism to elucidate the biological functions unique to trees (Xiao *et al*., 2009). Poplars are usually known as one of the most drought-sensitive woody plant groups, but their drought tolerance varies greatly among species, populations, and clones due to their great genetic diversity (Monclus *et al*., 2006).

The acclimation of plants to water deficit is the result of many different physiological and biochemical mechanisms. To gain a better understanding of drought stress acclimation and tolerance mechanisms in trees, we carried out an integrated morphological, physiological and biochemical analysis on the drought stress responses of Poplar clones Kranti under

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varied level of progressive water stress, especially for the seedling stage (up to 60 days after transplanting of cuttings). Poplar clone Kranti was selected to assess responses under drought at different levels of water stress, as it is a common clone used to be grown in Uttarakhand for making paper and plywood.

MATERIAL AND METHODS

Plant and growth conditions

The experiment was conducted at Department of Plant Physiology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Poplar clone Kranti, cuttings were raised by using uniform diameter and length (size 18-20cm) purchased from WIMCO seedlings (Pvt.) Ltd. Bagwala (Kashipur Road), Rudrapur, Udham Singh Nagar (Uttarakhand). The Poplar cuttings were planted in polybags for a period of 15 days. Sprouted cuttings were transplanted in earthen (diameter-30 cm and depth-30 cm), filled with fertile soil, i.e. soil texture was silty clay loam, pH-7.1, organic carbon- 0.86% and N, P, K- 245, 35.5 and 172 kg ha^{-1}.

Twenty-five pots were kept under control conditions (100% of field capacity) and 25 pots each under different levels of drought stress (75, 50 and 25% of field capacity). Transplanted sprouted cuttings were left for another 15 days for the initial establishment in earthen pots as a normal condition. Treatments were imposed after establishing sprouted cuttings. Drought levels were maintained in a different group of plants by keeping soil moisture status at 75, 50 and 25% of field capacity as compared to control. Thus control seedlings were also similarly grown in the earthen pots, except regular irrigation was allowed up to field capacity. Drought levels were maintained on the basis of CPE (Cumulative Pan Evaporimeter) reading taken from an agro-meteorological section of Crop Research Centre (CRC), G. B. Pant University of Agriculture & Technology, Pantnagar (Uttarakhand). The CPE dependent drought induction method is a standard method and regularly used in farm and pots for drought induction. To maintain different drought levels, the treated plants were irrigated with 2.5 L of water when the day for specific drought stage came, while alternate day to control plants. Water stress was imposed for a period of 60 days and physiological, growth and biomass responses of the plants were measured at specific time intervals.

Growth Parameters

At the end of the experiment (60 days), plant height, radial stem diameter and number of leaves of the seedling were determined and then a destructive harvest was carried out. Ten seedlings of Kranti for control and treatment were randomly sampled. The relative leaf water content (RWC) was determined as stated: 100×(FM-DM)/(TM-DM), where, FM is fresh mass, TM is turgid mass after re-hydrating the leaves for 24 hours at 4 ºC in darkness and DM is dry mass after oven-drying the leaves for 24 hours at 70 ºC. Dry weights were obtained by weighing the plant material after drying at 75 °C±3 unit, a constant mass reached. The harvest index was calculated by harvesting at least 10 seedlings of each treatment (n=10) as according to Michael *et al.* (1988).

Biochemical parameters

Free proline was determined by the method of Bates *et al.* (1973). The procedure of Heath and packer (1968) was followed for measuring the malondialdehyde content. Soluble sugar was measured as described by Mohsenzadeh *et al.* (2006), and the concentration was expressed as mg g^{-1} DW. Data management and statistical analysis were performed using SPSS software (SPSS, Chicago, IL, USA). Means were expressed with their standard error (±SE) and compared by ANOVA. All statistical tests were considered significant at $p \leq 0.05$.

RESULT AND DISCUSSION

Investigations on progressive drought stress are a very useful way to gain insight into the sudden or punctual responses to drought stress. In particular, the impact of progressive drought stress on plants should be assessed by examining drought effects during the time course using a wider range of water availability, since the physiological and biochemical processes of plants depend on the rapidity, severity, and duration of the drought event (Yang and Miao 2010).

Growth Parameters

The percent increase in height was higher in case of control plants as compared to treated plants at harvest stage, i.e. 60 days after stress. We observed a decline in the percent increase of height ca. 24 to 69% compared to control as we move from 75, 50 and 25% of FC of water stress. Up to 75%, FC of water stress decline was very less after that level of water stress decline in height was severe. The trend found for plant stem diameter was similar to that of plant height in drought-induced poplar plants (Table 1). Percent increase in stem diameter was higher in case of control plants as compared to that of water stress-treated plants and decline with the severity of water stress. The decline can be neglected up to 75% FC of water stress.

Table 1: Growth parameters of *Populus deltoides* L. clone Kranti seedling grown under various progressive water stress.

Characteristics	Control (% of FC) 100	Stressed (% of FC)			Decline over control (%)		
		75	50	25	75	50	25
Height (cm)	78.80	60.20	36.00	24.60	23.6	54.3	68.8
Radial stem diameter (cm)	9.1	7.2	6.6	5.1	21.1	27.9	44.0
Total biomass (g)	34.29	27.8	15.76	10.9	18.9	54.0	68.2
Harvest index	0.74	0.58	0.34	0.28	21.6	54.1	62.2

Both the economic and biological yields decreased with increasing levels of water stress. It was very less at the 75% of FC level of drought stress and after 50% of FC decrease was more than 50% at all the stage of sampling. Hence, harvest index (HI) also decreases with increasing levels of drought. We found a significant decrease in HI with increasing levels of drought stress.

The reduction was found to be 62% for Kranti clone of poplar when a severe drought was imposed (25% of FC irrigation water). The decline was very little in case of 75% FC of drought stress.

Due to water stress biomass synthesis decreased resulting in a lesser increase in height. Thus, drought affected overall phenology of poplar plants similar to certain other woody (Souch and Stephans, 1998; Calatayud *et al.,* 2000; Hasse *et al.,* 2000 and Kumari *et al.,* 2017) and crop plants as well. The shoot dry weight decreased progressively with increase in drought level.

The lesser radial diameter was due to a decrease in photosynthetic efficiency and fight of treated plants against water stress which is necessary for each metabolic process because any stress affects the normal functioning of plants. Souch and Stephens (1998) also have reported the loss in shoot height and radial stem growth under water stress compared to normal irrigated poplar plants.

Harvest index of the plant is an indication of how effectively dry mass is translocated into useable above ground components. With the increased level of drought, there was a significant decrease in harvest index. Hence, a decrease in harvest index occurred due to non-availability of adequate water which influenced both economical yield and biological yield (Kumari, 2015).

Relative water content (RWC) decreased with the severity of drought and period of exposure to plant against water stress (Table 2). Percentage values of RWC vary from 84 - 81, 84 - 72, 84 - 61 and 84 - 57% of control, 75, 50 and 25% FC of drought stress respectively at 15, 30, 45 and 60 days after imposing drought stress. In RWC also Decline can be neglected up to 75% FC of water stress.

The leaf relative water content directly reflects the water status of plants. In our study, the results showed that drought stress significantly affected leaf relative water content, and stem height showed significant negative correlations with RWC under water-stressed treatment. Similar results have been reported in previous studies on poplars (Liang *et al.,* 2006, Yang and Miao 2010).

Table 2: Relative water content (RWC) and % decline in relative water content over control of *Populus deltoides* L. clone Kranti seedling grown under various progressive water stress

Drought levels in terms of FC (%)	Relative Water Content (RWC) %					% Decline over to their respective control			
	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	15 DAS	30 DAS	45 DAS	60 DAS
Control	83.51	82.57	81.10	80.89	80.67	-	-	-	-
75	83.52	74.80	73.27	72.17	71.60	9.41	9.66	10.78	11.24
50	83.54	68.07	65.97	64.95	60.50	17.56	18.66	19.71	25.00
25	83.51	64.77	60.07	59.32	56.73	21.56	25.93	26.67	29.67

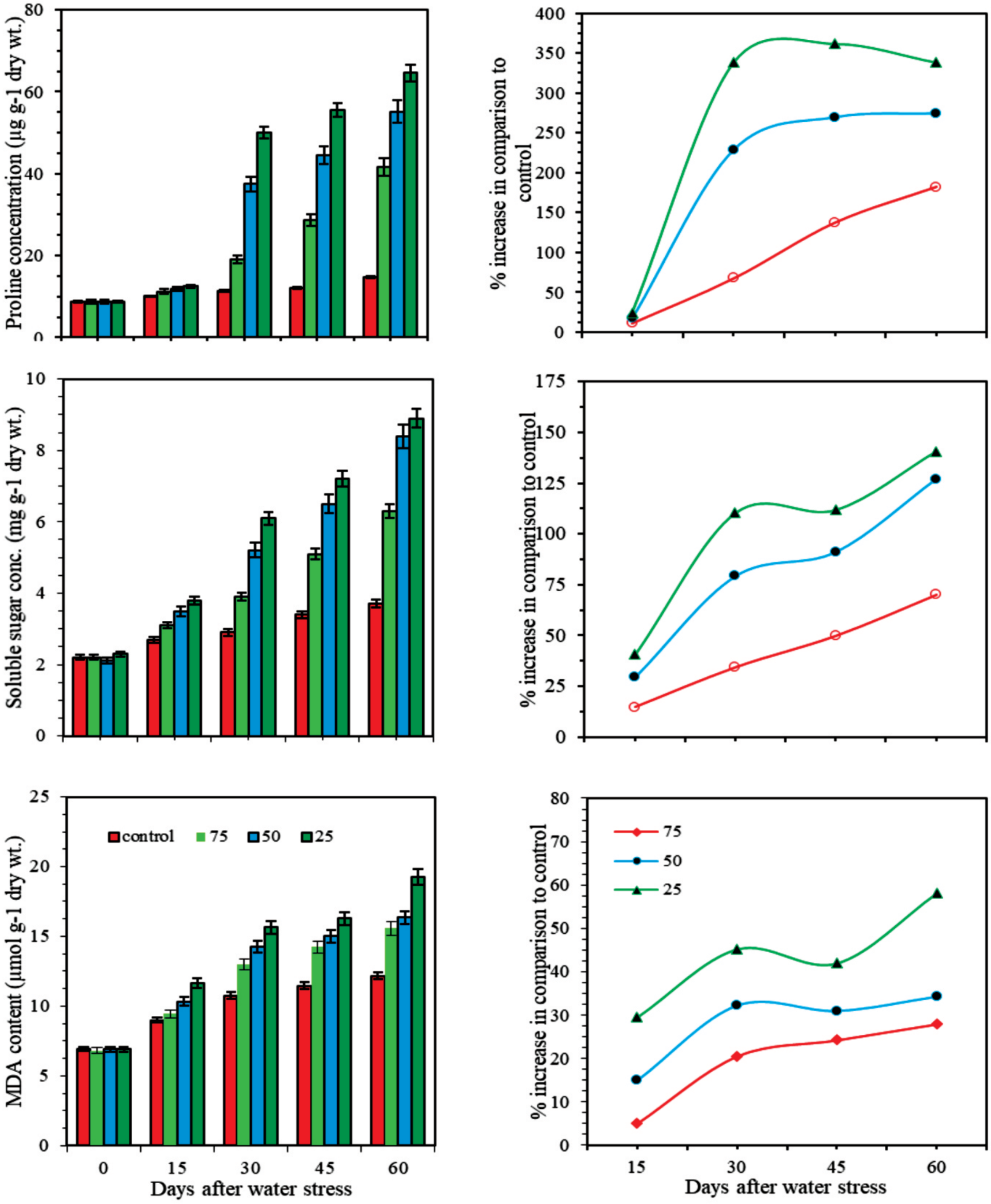

Fig. 1: (A) Proline concentration, (B) % increase in proline concentration over control, (C) soluble sugar concentration, (D) % increase in soluble sugar concentration over control, (E) malondialdehyde (MDA) content, (F) % increase in malondialdehyde (MDA) content over control in Kranti clones of poplar seedlings grown under various progressive drought stress at different time after imposing drought stress.

Biochemical parameters

A significant and positive correlation between proline accumulation and drought severity was observed. In the present experiments, proline content increased as the drought level increased from 75 to 25% of FC in Kranti clone of *Populus deltoides*. Proline accumulation increased many folds in drought stress-treated plants (75, 50 and 25% of FC) as compared to control. However, this fluctuation was less dramatic in the non-treated plants. Lowest proline accumulation was observed in plants maintained at 75% of FC of drought level after every stage of sampling (Fig.1). Highest proline accumulation was detected in plants treated with 25% of FC of drought level. The proline concentrations were 41.59, 55.10 and 64.54 μg g^{-1} dry wt. at 60 days of imposing drought stress for 75, 50 and 25% of FC of drought stress, respectively. Lowest proline content was obtained in non-treated plants (14.73 μg g^{-1} dry wt.) before imposing stress.

Compared with the well-watered cuttings, significant increments in the concentrations of soluble sugar occurred at 75, 50 and 25% of FC. At the end of the experiment, compared with the well-watered cuttings, the increments of soluble sugar in the water-stressed cuttings were 70, 127 and 141% for 75, 50 and 25% of FC of drought stress respectively at 60 days after imposing stress. Accumulation was lesser in the case of plants at 75% of FC.

MDA content was significantly influenced by induction of drought stress. The leaves of poplar clone showed about 11.46 - 16.27 μmol g^{-1} dry wt. at 45 days and 12.17 - 19.24 μmol g^{-1} dry wt. after 60 days of imposing drought stress, respectively, as we move from control to 25% of FC of drought stress. MDA content increased with increased level and duration of drought exposure.

Plant growth is responsive to progressive drought stress, and the reactions depend on the adaptation to the rapidity, severity, duration of the drought event (Yang and Miao 2010). Drought stress significantly inhibited growth, decreased the Plant height, stem radial diameter, Plant biomass, harvest index and RWC, increased the relative electrolyte leakage and MDA content, and, at the same time, induced the accumulation of soluble sugars and free proline in the Poplar plants of clone tested. Physiological and biochemical changes at the cellular level that are associated with drought stress typically include a reduction in plant growth and accumulation of osmolytes and MDA.

The plant's defense against drought stress requires osmotic adjustment, which, to a certain degree, can be achieved through a synthesis of intracellular solutes (Yang and Miao 2010). Proline may protect protein structures by maintaining their structural stability (Bates *et al.,* 1973), and, accordingly, drought stress significantly increases proline accumulation (Sofo *et al.,* 2004, Ren *et al.,* 2006). Soluble sugars acting as osmoprotectants stabilize proteins and membranes, most likely substituting the water in the formation of hydrogen bonds with polypeptide polar residues (Crowe *et al.,* 1992) and phospholipid phosphate groups (Strauss and Hauser 1986). As one of the end products of lipid peroxidation, the MDA content reflects the degree of the peroxidation of membrane lipids (Taulavuori *et al.,* 2001). The MDA contents significantly increased with drought stress progressed in the poplar clones, but MDA possessed negative effects on drought tolerance. The significant increase of MDA contents with drought stress progressed in the two poplar species suggested drought stress caused oxidative damages in poplar clones, similarly as detected in poplar trees (Yang and Miao 2010) olive trees (Sofo *et al.,* 2004), and *Coffea arabica* (Queiroz *et al.,* 1998).

CONCLUSION

When the cuttings were exposed to progressive drought stress, punctual changes appeared earlier in height growth and stem diameter inhibition, relative water content, free proline and sugar contents and MDA content. Our results manifested that acclimation to drought stress is not only with the environmental factors of plant's natural habitats but also related with the rapidity, severity, duration of the drought event and their interaction. Different responses to different field capacity in poplar clones improved our understanding of the mechanisms that enable plants to survive under different drought stress. Secondly, we can emphasize with our experiment that poplar plants can maintain their better growth and biomass only up to 75% of FC after that stress shows its severity so much that the aim of plants is only to survive, and biomass maintenance become vague.

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Citation:

Kumari A, Singh AK, Singh SK and Khan IM. 2018. Morphological, physiological and biochemical responses of poplar plants to drought stress. *Journal of AgriSearch* **5**(3): 184-189