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EFFECT OF WATER STRESS ON NITRATE REDUCTASE ACTIVITY AND GROWTH PARAMETERS OF SOME *DALBERGIA SISSOO* ROXB. CLONES UNDER GLASS HOUSE CONDITION

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Abstract: *Dalbergia sissoo* is one of the most important timber species of north India and also known as strong biological nitrogen fixer through its root nodules. Its growth and development is highly influenced by the availability of water. Therefore, an attempt was made to study the effects of different watering schedules i.e. daily, weekly, fortnightly and monthly on the growth, biomass production, and nitrate reductase (NR) activity in leaves under pot culture condition. The experiment was conducted during the month of May and the response of four different watering schedules *i.e.* daily, weekly, fortnightly and monthly in twenty elite clones of *Dalbergia sissoo* collected from Uttar Pradesh, Haryana, Punjab, Rajasthan and Uttarakhand was studied. It was observed that growth, survival and nitrate reductase activity of leaves were adversely affected with the increase of water stress treatments. Monthly watering seedlings were severely affect as compared to weekly and fortnightly watering schedules. However, two clones collected from Uttar Pradesh (2, Bijnor and 51, Gonda) one each from Haryana (57, Ambala) and Rajasthan (97, Sriganganagar) performed somewhat better than all clones studied.
Keywords: *Dalbergia sissoo*; Morphology; Nitrate reductase activity; Watering schedules
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INTRODUCTION

Dalbergia sissoo is an important timber and nitrogen fixing tree species. It is best known as a premier timber but is also utilized as fuel wood as well as for shade and shelter. It is found in tropical to subtropical climate in natural and planted forest, mainly along with forest margins near streams and rivers, agricultural areas and roadsides (Duke, 1983; Sharma *et al*., 2000; Langeland and Stocker, 2001). The growth and development of *Dalbergia sissoo* is highly influenced by the availability of water. In the absence of water availability the plant becomes unable to absorb nutrients from the soil which are essential for their growth and development. Decrease in soil water availability affects the rate of diffusion of many plant nutrients. Since *Dalbergia sissoo* is known for its biological nitrogen fixation activity and water stress can decrease absorption of plant nutrients which directly affects to the various metabolic and growth process. Nitrogen assimilation is influenced by several environmental factors and positively correlated with soil moisture and temperature and inhibited by the dry condition as well as in extreme cold (Jordan *et al*., 1978; Stewart *et al*., 1978; Jaiger and Werner, 1981).

The objective of the present study was to assess the water stress effect on some selected clones collected from U.P., Haryana, Rajasthan, Punjab and Uttarakhand of *Dalbergia sissoo* in relation to NRA and growth parameters. The findings will thus be useful in developing a technology for protecting the seedlings from desiccation in the field and to find out the best performing clonal material after screening them with reference to water stress.

EXPERIMENTAL

Vegetative propagated plant material in polythene bags was procured from the Genetics and Tree Propagation Division, FRI, Dehradun. These plants were transplanted into earthenware pots (30 cm diameter) filled with a mixture of soil, sand and farmyard manure in the ratio of 2:1:1. All the seedlings were kept under glass house condition in Plant Physiology, Botany Division, Forest Research Institute, Dehradun. The details of the clones are given below (Table 1). Plants from each clone number were divided into four groups and subjected to four different water stress treatments viz., daily (control), weekly, fortnightly and monthly watering intervals in the month of March. Morphological observations were recorded from the month of May after the plant established well. The various growth data i.e. height, collar diameter, root length, number of leaves and branches, survival percentage and fresh and dry weight were recorded. For nitrate reductase (NR) activity leaf samples were collected from all the clones, washed thoroughly in tap water followed by distilled water and finally excess of moisture was removed with the help of tissue paper.

Table 1. Clones of *D. sissoo* for different water schedule

#	Clone no.	Collection site
1.	2	Bijnor, Uttar Pradesh
2.	5	Bijnor, Uttar Pradesh
3.	6	Bijnor, Uttar Pradesh
4.	9	Haridwar, Uttarakhand
5.	13	Haridwar, Uttarakhand
6.	14	Haridwar, Uttarakhand
7.	18	Saharanpur, Uttar Pradesh
8.	19	Saharanpur, Uttar Pradesh
9.	20	Saharanpur, Uttar Pradesh
10.	23	Saharanpur, Uttar Pradesh
11.	49	Gonda, Uttar Pradesh
12.	51	Gonda, Uttar Pradesh
13.	57	Ambala, Haryana
14.	97	Sriganganagar, Rajasthan
15.	138	Hoshiarpur, Punjab
16.	201	Gonda, Uttar Pradesh
17.	218	Gonda, Uttar Pradesh
18.	237	Gonda, Uttar Pradesh
19.	1003	F.R.I., Dehradun, Uttarakhand
20.	5021	Sangrur, Punjab

The leaf tissue collected from individual replicates was chopped into small pieces of about 2-3 mm in length and width. Approximately 500 mg of the chopped parts were taken in a flat bottom culture tubes (30 ml capacity), containing 3 ml phosphate buffer (0.2M KH_2PO_4, pH 7.5) and 3 ml substrate (0.2M KNO_3) and embedded in ice trays. The buffer and substrate strength for this species was already standardized (Pokhriyal *et al*., 1988). These tubes were evacuated with the help of vacuum pump (Metrex, Rotary High Vacuum Pump) for about 2 minutes. The process was repeated until the plant tissues were fully submerged into the incubation medium. These tubes were transferred to shaking incubator at 30ºC in dark. The tubes were removed after one hour and immersed into a boiling water bath for 4 minutes to stop the reaction and effective removal of the nitrate accumulated in the plant tissue. The same method was adopted for nitrate reductase (NR) (E.C. 1.6.6.1) activity as earlier described (Klepper *et al*., 1971) with some modifications (Nair and Abrol, 1977).

End product, the amount of nitrate reduced into nitrite during enzyme activity was determined by the method earlier described (Evans and Nason, 1953). A required amount of an aliquot was pipette in a clean test tube, 1 ml sulphanilamide (1%, sulphanilamide in normal hydrochloric acid) was added, and shaken well, followed by 1 ml of NEDD (0.01% N, 1-Nepthylethylene diamine dihydrochloride) and mixed thoroughly. Colour was allowed to develop for 25 minutes and final volume was made 6 ml with the help of distilled water. A change in colour intensity was estimated at 540nm in spectrophotometer immediately.

Table 2. Environmental parameters recorded in glass house as well as outside during January to December 2013.

Month	Min. temperature (ºC)		Max. temperature (ºC)		R. Humidity (%)	
	Inside	Outside	Inside	Outside	Inside	Outside
January	9.91	2.5	18.95	19.8	96.91	99.0
February	15.67	7.7	23.47	21.0	88.22	97.0
March	22.0	10.6	30.19	27.4	80.27	92.0
April	21.92	13.3	34.5	31.8	71.57	76.0
May	21.58	17.3	37.67	36.2	59.91	64.0
June	23.57	22.1	38.05	31.0	61.40	92.0
July	24.58	23.4	35.76	30.3	64.0	95.0
August	25.12	22.6	36.47	30.2	62.10	96.0
September	25.88	20.2	35.78	30.6	64.94	94.0
October	23.10	16.7	29.37	28.5	72.0	95.0
November	13.12	7.5	23.15	24.7	83.75	98.0
December	10.31	4.4	21.24	21.7	92.15	100.0

RESULTS AND DISCUSSION

On the basis of individual clone performance, in daily watering maximum height (cm) was observed in clone no. 5 (114.00±9.54), 51 (110.33±3.93) and 2 (107.00±1.15); whereas, in weekly watering clone no. 5 (98.67±3.93) performed best followed by clone no. 51 (97.00±1.00) and 97 (92.67±4.10). Clone no. 97 showed better results as compare to clone no. 5 (91.00±4.16) and 51 (81.33±3.38) in fortnightly watering. However, in monthly watering, clone no. 5 (82.33±3.51) performed better as compared to clone no. 2 (76.00±2.00) followed by 51 (72.66±1.45) and 97 (72.33±2.85) (Figure 1a).

In collar diameter (mm) of daily watering clone no. 138 (10.61±0.88) showed better performance followed by clone no. 97 (8.78±0.54) and 5 (8.25±0.39). Whereas, in weekly watering clone no. 97 (7.81±0.70), 57 (7.49±0.56) and 51 (7.16±0.96) performed better than all. In fortnightly watering clone no. 97 (7.00±0.62) gained higher collar diameter than 57 (6.21±0.79) and 51 (6.03±0.50). However, in monthly watering clone no. 97 (6.08±0.21) gained higher diameter than 57 (5.17±0.54) and 5 (4.92±0.55) (Figure 1b).

Within different clones maximum root length (cm) was recorded in clone no. 18 (66.33±1.45), 2 (64.33±1.20) and 218 (58.67±3.71) respectively in daily watering schedule whereas, in weekly watering clone no. 218 (58.33±10.97), 2 (54.67±2.40) and 13 (54.00±2.31) performed well. In fortnightly watering clone no. 138 (51.33±1.45), 18 (49.67±6.64) and 13 (49.33±3.76) performed better whereas, in monthly watering clone no. 13 (43.67±5.21), 2 (43.00±2.08) and 18 (39.67±1.45) performed well (Figure 1c).

In branching pattern, clone no. 2 (5.33±0.88) and 57 (4.67±0.67) performed higher number of branches in daily watering whereas, in weekly schedule clone no. 2 (4.00±0.58) and 51 (4.33±0.58) showed higher values. In fortnightly and monthly watering clone no. 2 (2.67±0.33), 57 (2.67±1.20), 18 (2.00±0.00) and 2 (1.67±0.33) produced minimum branches (Figure 2a).

Highest leaf number in daily watering was shown by clone no. 57 (176.67±3.53) followed by 2 (175.00±2.89) and 138 (170.00±2.89). In weekly watering schedule, clone no. 138 (105.00±11.02) performed better than 51 (95.33±20.20) and 57 (93.00±1.15). However, clone no. 138 (84.00±1.53) produced maximum number of leaf as compare to 57 (84.67±4.51) and 5 (53.33±3.06) in fortnightly watering schedule. In monthly watering maximum values were observed in clone no. 57(40.00±7.21) followed by 97 (18.00±4.36) and 218 (18.00±2.52) (Figure 2b).

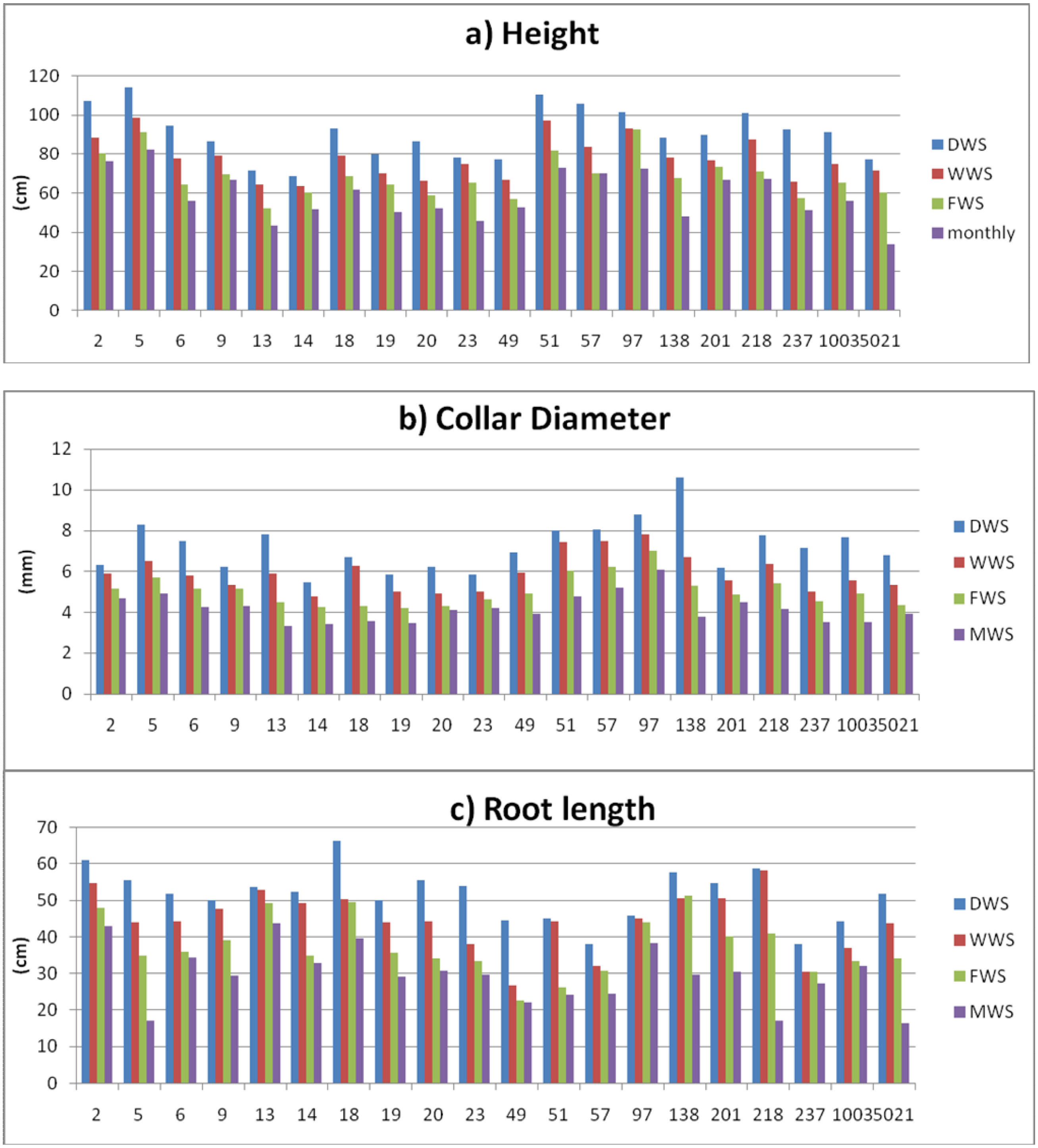

Figure 1: Height, collar diameter and root length of *D. sissoo* clones under different watering schedules (DWS: daily watering schedule (WWS: weekly watering schedule, FWS: fortnightly watering schedule and MWS: monthly watering schedule).

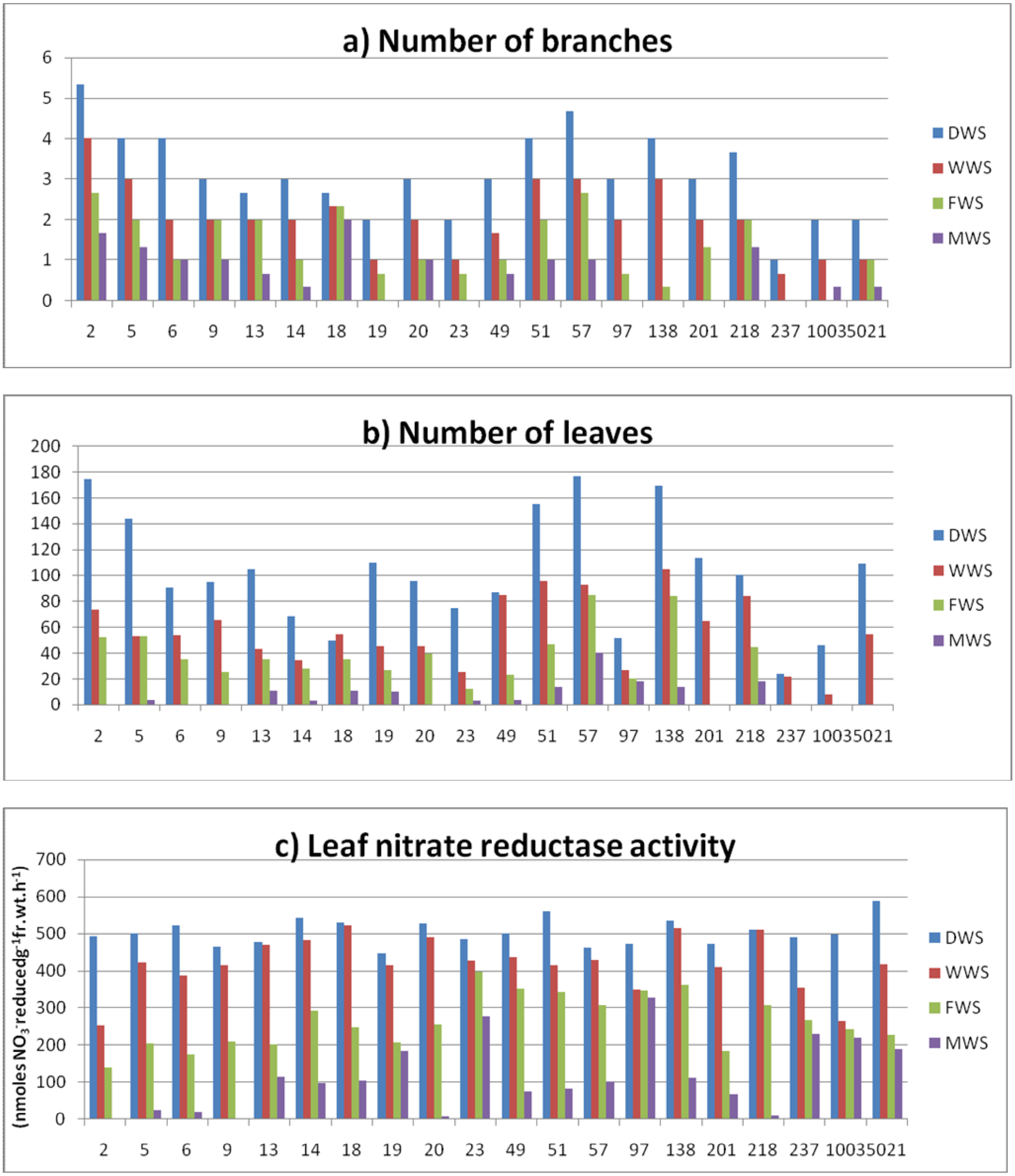

Figure 2: Number of branches, number of leaves and nitrate reductase activity in leaves of *D. sissoo* clones under different watering schedules (DWS: daily watering schedule, WWS: weekly watering schedule, FWS: fortnightly watering schedule and MWS: monthly watering schedule).

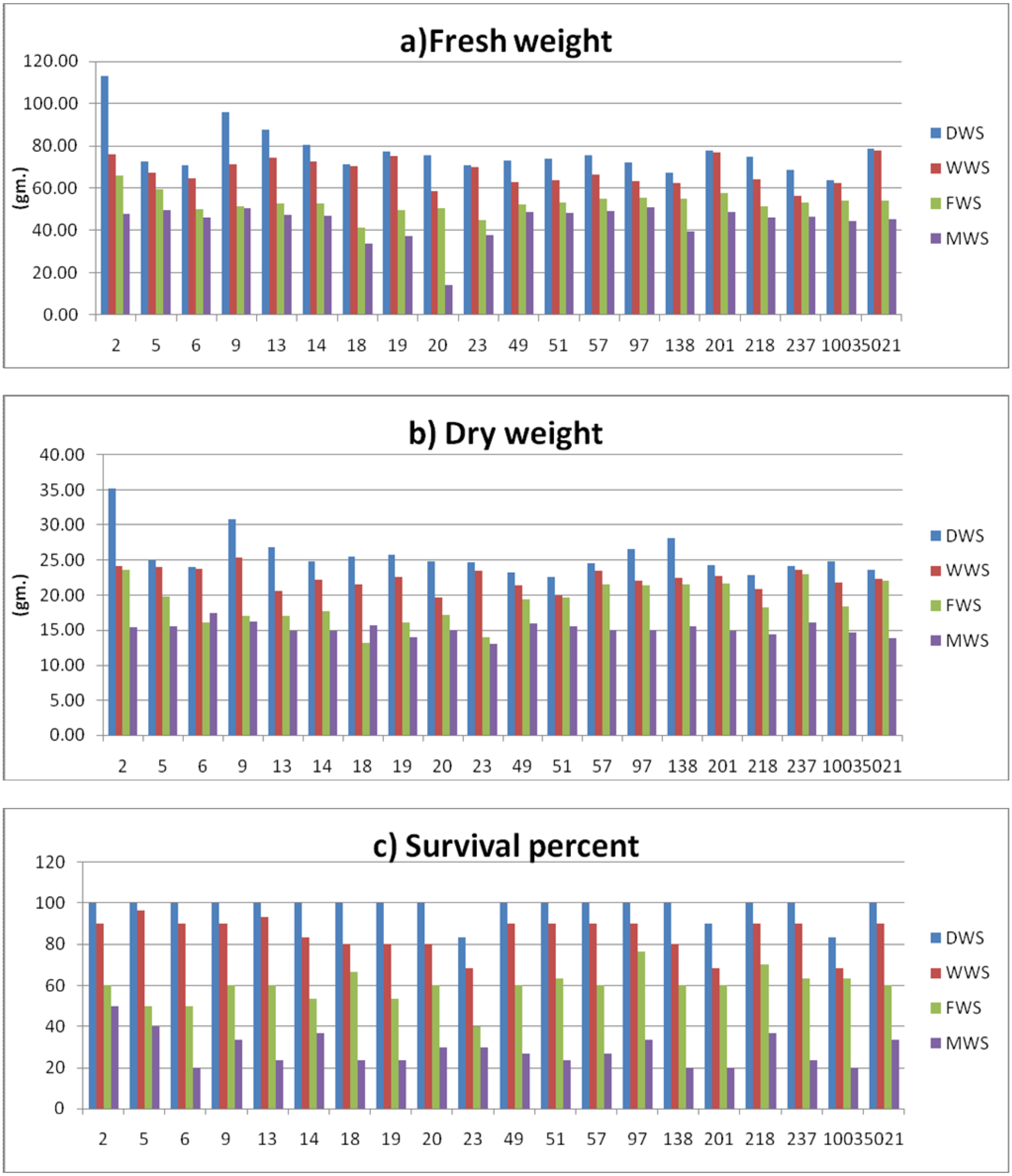

Figure 3: Fresh weight, dry weight and survival percentage of *D. sissoo* clones under different watering schedules (DWS: daily watering schedule, WWS: weekly watering schedule, FWS: fortnightly watering schedule and MWS: monthly watering schedule).

When plant leaves were assessed for nitrate reductase (nmoles NO_3^-reduced g^{-1} fr. Wt. h^{-1}) activity for daily watering schedule, clone no. 5021 (587.91±45.54) showed highest NR activity followed by clone no. 51 (559.35±25.94) and 14 (542.35±19.13); whereas, in weekly watering clone no. 18 (522.34±16.59) showed higher activity than 138 (514.22±35.41) and 218 (510.00±41.70). Clone no. 23 (396.81±11.70) performed best in fortnightly watering followed by 138 (361.70±49.21) and 49 (353.19±29.69). In monthly watering clone no. 97 (328.51±22.45) could perform best in terms of NR activity followed by 23 (278.09±96.38) and 237 (229.79±81.89) (Figure 2c).

Maximum fresh weight (gm) per plant in daily watering was observed in clone no. 2 (112.80±6.93) followed by 9 (95.75±2.29) and 13 (87.67±1.20); whereas, in weekly watering it was higher in 5021 (77.77±1.34), 201 (76.77±1.08) and 2 (76.22±8.67). Clone no. 2 (65.90±1.73) shown higher plant biomass as compare to 201 (57.48±1.13) and 97 (55.39±0.62) in fortnightly watering schedule. However, in monthly schedule clone no. 97 (51.17±0.62) showed higher values as compared to 9 (50.47±3.47) and 5 (49.50±1.69) (Figure 3a). In case of dry weight higher values were observed in clone no. 2 (35.23±2.89), 9 (30.85±4.04) and 13 (26.80±0.95) respectively in daily watering. Whereas, in weekly watering clone no. 9 (25.33±1.13), 2 (24.20±2.89) and 5 (24.06±1.33) were observed higher dry weight as compared to other. In fortnightly watering higher biomass was recorded in clone no. 2 (23.60±2.31) followed by 237 (23.04±1.71) and 5021 (22.70±1.55) and in monthly watering it was observed maximum in clone no. 6 (17.36±1.23), 9 (16.23±3.47) and 237 (16.04±1.19) (Figure 3b).

The survival percentage of all the clones decreased significantly with increasing level of water stress and also decreased in the soil moisture content (Figure 3c). The survival percentage of all the clones was recorded between 80-100%, 68-90%, 40-70% and 20-40% for daily, weekly, fortnightly and monthly watering schedule at the soil moisture content of 31.76%, 8.15%, 1.1% and 0.08 % respectively. Moreover, when seedlings of *Dalbergia sissoo* were subjected to severe water stress (monthly watering schedule), that sudden shock reduced the elasticity or resistance to drought and mortality rate is increased. Some times during planting, only few hours of exposure may cause a significant decrease in survival (Hermann, 1967, Coutts, 1981, Tabbush, 1987). When drought induced it is more important for plant to survive than for high growth rate. Under daily watering schedule 100% survival was observed. The growth parameters in *Dalbergia sissoo* were significantly influenced by different watering schedules.

From the month of January onwards to May, temperature increased from 9.91 to 21.58 under glass house conditions. However, from June to September there were not much differences and after that the inside and outside temperature decreased gradually. In winter months the inside temperature always remain slightly higher. In case of maximum temperature there were not much differences from the month of January to May and after that from May to September the inside temperature remained always higher. The inside relative humidity remained between 59.91 to 64.94 % whereas; minimum relative humidity was recorded in the month of May. In rest of the months there were no much difference observed (Table 2). Looking on the table no. 2 May and June are the hottest and dryer months. During these months plants have to survive in these harsh conditions. After the month of October again there were no much differences between inside and outside temperature.

As a consequence of completion and differential capacity for draught tolerance tree species are often segregated along moisture gradients. The structural and physiological adaptations associated with draught tolerance of plants are numerous and diverse (Pallardy, 1981) and they have seldom received detailed study in woody species that vary in natural distribution. Initial growth is an important factor in the subsequent survival and development of individual tree species (Nautiyal *et al*., 1994). In the field, it is not only the absolute value of water deficiency that can be tolerated, but also the response to continuously changing soil moisture that is important for survival (Mazzoleni and Dickman, 1988). The water stress or draught has profound effect on growth, yield and plant quality. The first effect of the stress may well be a loss of turgor that affects the rate of cell expansion and ultimate cell size. Loss of turgor is most probably the process of most sensitive to water stress. The result is the decrease of growth rate, stem elongation, leaf expansion and narrowing of stomatal aperture (Nautiyal *et al*., 1996).

This study clearly showed that the seedlings of *Dalbergia sissoo* could sustain the water stress up to seven days interval. Similar results were reported in the *Grevillea robusta* (Nautiyal *et al*., 1996). A considerable reduction in biomass and nitrogenase activity due to water stress treatment in *Albizia procera* was also reported (Pokhriyal *et al*., 1989). The uptake of nitrogen was also decreased under water deficit (Sharma, 1982). The results of these findings support many other findings that show a reduction of growth before the wilting point is reached (Handson and Hits, 1982; Van Volkenburgh and Boyer, 1985; Goubin and Kemp, 1992; Wang *et al*., 1992; Nautiyal *et al*., 1993). It is apparent that the relation between water stress and growth is very complex, involving many physiological processes. Some investigators claim that the most serious effect of draught is a reduction of photosynthetic surface and of dry matter production. However, the reduction in net photosynthetic rate per unit of surface is also important for the growth and development.

Table 3. ANOVA for different parameters

Parameters	S.E.M	p value	F value	Significance
Height	1.141	0.00	57.01	S
Collar diameter	0.104	0.00	102.45	S
Root length	0.68	0.00	25.09	S
Number of branches	0.08	0.00	512.36	S
Leaf number	2.54	0.00	72.12	S
NR activity in leaf	9.083	0.00	144.56	S
Fresh weight	0.81	0.00	213.32	S
Dry weight	0.29	0.00	242.58	S
Survival	1.56	0.00	87.54	S

CONCLUSION

Vegetatively propagated plant material of *Dalbergia sissoo* collected from Uttar Pradesh, Haryana, Punjab, Rajasthan and Uttarakhand were subjected to four different watering schedules *i.e.* daily, weekly, fortnightly and monthly intervals under the glass house condition. A marked reduction in morphological and other parameters was observed with the increase in stress of watering treatments. The effect of water stress in monthly watering schedule shown prominent effect as the plant survival rate, nitrate reductase activity was very low and the seedlings sustained water stress up to the one week interval. A clear reduction was observed in case of height and collar diameter between every watering treatment. All the parameters studied were observed statistically significant at 0.05 levels. On the basis of all parameter studied and overall performance of the clones, it was observed that clone number 2 (Bijnor, U.P.), 51 (Gonda, U.P.), 57 (Ambala, Haryana) and 97 (Sriganganagar, Rajasthan) performed best among all.

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