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Effect of salinity and population variability on physiological parameters for salinity tolerance in teak (*Tectona grandis* L.)

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Abstract
Teak is one of the highly demanded and planted tree species throughout the world. Teak (*Tectona grandis* L.) belongs to Lamiaceae family which is also known as "King of Timber" due to its wood quality and multifarious uses. Now a days, soil salinity is increasing across the globe due to climate change and sea level raising. There is an urgent need to identify the good genotypes or populations of Teak which are tolerant to soil salinity. Hence, the present research study was carried out in Green House Nursery at College of Forestry, Navsari Agricultural University, Navsari. The seed material of Teak collected from five different populations *i.e.*, Bhenskatri, Junagadh, Padamdungari, Subir and Waghai. Seeds were sown in Forest Nursery and grown upto two months in pots before starting the salinity treatments. Thereafter, four salinity treatments along with a control was induced through irrigation with a range of diluted sea water (EC- 1 dS/m, 2 dS/m, 4 dS/m and 6 dS/m). The study revealed that there was large variation for physiological traits among all the population, salinity levels and their interaction effect. It was found that the physiological parameters of Teak in ontogeny stage was reduced when the salt concentration increased. In addition, Junagadh and Bhenskatri population was performed better among all the five populations for studied traits at all five salinity levels. However, all five populations of Teak were survived under continuous supply of saline water and the detrimental effect of salinity was evident at higher dose (EC ≥ 4 dS/m). It is interesting to see that Junagadh and Bhenskatri population does not compromise for physiological traits under salinity stress which shows resistance against salinity. Thus, Junagadh and Bhenskatri population was recommended for plantation purposes in the salt affected areas in south Gujarat region of India. Population variability should be effectively captured for further tree improvement of teak for salinity resistance.

Keywords: Teak, salinity, teak populations, salt affected areas, tree improvement, plantations

Introduction
Approximately 800 million hectares land of the world are affected by salinity which accounts about 6% of the world's surface area. Now a days, salinity in soil is becoming one of the important environmental limiting factors that limit plant growth and development (Munns and Tester 2008; Golldack *et al.* 2014) [16, 10]. Moreover, saline land is rapidly increasing due to the effect of climate change and raising sea level. Therefore, there is an urgent need to develop salt tolerant tree species (Munns *et al.* 2012) [17] which entails a comprehensive understanding of the plant's response mechanism in high salinity conditions (Bartels and Sunkar 2005) [1]. Salinity in soil can cause several challenges for plant's ontogeny including water stress, physiological stress, malnutrition and accumulation of excess ions to potentially toxic levels. There are three main salinity tolerance mechanisms proposed by Munns and Tester (2008) [16]. In ion exclusion mechanism, the net exclusion of toxic ions comes from the shoot of the plant. In tissue tolerance mechanism, the compartmentalization of toxic ions comes into specific tissues, cells and subcellular organelles of the plant. In shoot ion-independent tolerance mechanism, the maintenance of growth and water uptake independent of the extent of Na^+ accumulation comes in the shoot of the plant. There are several other physiological components likely to contribute to salinity tolerance in plants.

In addition, a large number of factors can influence plants' responses to salinity due to a very complex nature of salinity tolerance in plants (Gupta and Huang 2014) [13].
In regards to salinity, in the first phase, *effects of salinity on water relations* causing stomatal closure and the inhibition of leaf expansion (Munns and Termaat 1986) [15] and in second phase, *the ion-dependent response to salinity* develops over a longer time of period and involves to build-up of ions in the shoot to toxic levels which is causing premature senescence of leaves that reduced growth and yield or even death of the plant (Munns and Tester 2008) [16]. Furthermore, salt tolerance varies among and between genotypes/ species/ varieties and ontogenetic stages which is usually evaluated by a significant reduction in growth, biomass, yield or survival rates (Carillo *et al.* 2011) [2]. Such responses of species to salinity are widely documented in grass species and annual agricultural crops (Gulzar and Khan 2006) [12]. However, information regarding to forest tree species to salinity tolerance is limited in literature. The salt tolerant forest tree species can be used in salt affected waste land for restoration through mass plantations. Henceforth, the selection of salt-tolerant genotypes of forestry species is most important from an environmental as well as economic point of view.
Teak is one of the most valuable timber tree species and mostly planted tree species in India as well as in abroad. Teak (*Tectona grandis* L.) is a deciduous forest tree species which belongs to Lamiaceae family (Dhaka 2016) [6]. Its wood is useful for multifarious utility such as sawn timber of veneer, agricultural implements, for furniture making, for shipbuilding and many more uses (Dhaka and Jha 2017) [4]. Therefore, Teak is recognized as "King of Timber" due to the durability, workability and aesthetic property of wood which accounted it as one of the most demanded timber tree species in the national and international market (Dhaka and Jha 2018) [5]. Notwithstanding, there is a great importance of this species, a little scientific study is available on the natural population variability for response to salinity tolerance in Teak. Population variability studies in any tree species are important for selection of genotypes which is the base of genetic improvement. Hence, the present experiment was conducted to investigate the effect of salinity levels and population variability on the physiological parameters to identify the salinity tolerant genotypes in Teak.

Methods and Materials

Experimental Location

The present research study was carried out in Forest Nursery under controlled conditions at College of Forestry, Navsari Agricultural University, Navsari. The study was carried out in a green net house (air humidity ≈ 70 per cent) under controlled conditions of temperature (max 25±3 °C; min 10±3 °C), and 1000 μE $m^{-2}s^{-1}$ of light intensity. The seed material of Teak collected from five different populations *viz.*, Bhenskatri, Junagadh, Padamdungari, Subir and Waghai (Figure 1) and sown in Forest Nursery. Plants were grown for 2 months in pots before starting the salinity treatments with regular watering. After that, salt stress of different salinity levels was imposed through irrigation with a range of diluted sea water (EC 1 dS/m, 2 dS/m, 4 dS/m and 6 dS/m). The EC of control treatment was controlled by supplying 0.2 dS/m EC diluted sea water to avoid any fluctuation in natural tap water. Pots were flooded to mostly two cm below the soil surface because of that the capillary movement of water which ensured all of the soil volume was saturated. The physiological parameters *viz.*, net photosynthetic rate (μ mol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}) and transpiration rate (mmol m^{-2} s^{-1}) were measured with the help of InfraRed Gas Analyzer (Photosynthetic Meter, Model CI-340, Handheld Photosynthetic System, CID-Bioscience). Every observation was recorded between 8:30 to 10:30 am on monthly basis.

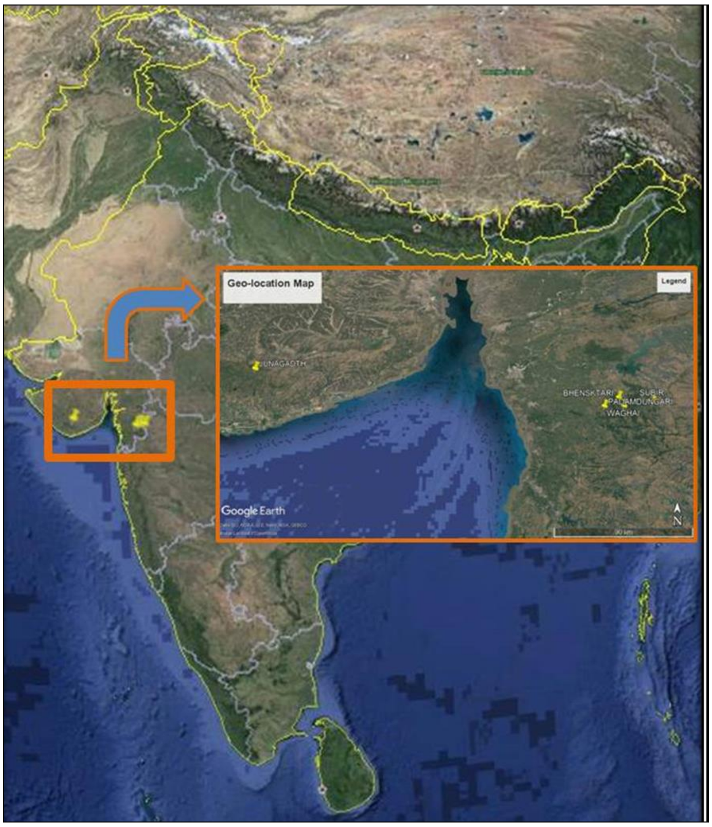

Fig 1: Geo-locations of the different populations of Teak from the south Gujarat region of India

Statistical Analysis

The experiment was laid out in Forest Nursery under controlled condition with factorial concept considering salinity levels as treatment factor and population as genotype factor.

$$Y_{ijk} = \mu + T_i + G_j + TG_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} is the analysed variable, µ is the general mean, T_i is the treatment effect (fixed), G_j is the genotype effect (fixed), TG_{ij} is the interaction of the treatment with the genotype, and ε_{ijk} is the residual. The results for each level were accepted as statistically significant if $P < 0.05$. Furthermore, to test the mean differences between the treatments, post-hoc comparison was performed using the LSD test in SAS software as per suggested by Gomez and Gomez (1984) [11].

Experimental Results

Effect of salinity levels and population source on net photosynthetic rate of Teak

In the present research study, the net photosynthetic rate varied significantly under the influence of salinity stress. The analysis of variance showed significant effect of salinity levels and populations for net photosynthetic rate trait. At the end of experiment, the maximum net photosynthetic rate (6.34 umol/m^2/s) observed at 0.2 dS/m whereas, the minimum net photosynthetic rate (0.98 umol/m^2/s) observed at 6 dS/m. The net photosynthetic rate decreased when salinity level increased. The population also showed significant variation for salinity tolerance in Teak. The maximum net photosynthetic rate found in Junagadh population (5.95 umol/m^2/s) while, the net photosynthetic rate observed in Subir population (1.54 umol/m^2/s) in Teak (Table 1).

Table 1: Main Effects of population and salinity level of irrigation water on net photosynthetic rate (P_n) in five Teak populations grown at five levels of saline water upto 120 days

Treatments	P_n (umol/m^2/s)			
	30 days	60 days	90 days	120 days
Population				
Bhenskatri	10.11	7.48	6.75	4.60
Junagadh	7.01	5.13	7.43	5.95
Padamdungari	6.63	3.86	4.57	3.52
Subir	3.17	5.54	1.92	1.54
Waghai	5.65	7.20	2.21	1.76
Salinity level				
0.2 dS/m	9.52	11.21	9.06	6.34
1 dS/m	7.89	7.66	6.49	4.49
2 dS/m	6.78	4.91	3.74	3.41
4 dS/m	4.73	3.53	2.37	2.15
6 dS/m	3.66	1.90	1.22	0.98
$LSD_{0.05}$				
Population	0.59	0.67	0.51	0.39
Salinity level	0.59	0.67	0.51	0.39

In the present research study, the net photosynthetic rate varied significantly under the interaction effect of population and levels of salinity. At the end of experiment, the maximum net photosynthetic rate (11.37 umol/m^2/s) observed at 0.2 dS/m control in Junagadh population whereas, the minimum net photosynthetic rate (0.25 umol/m^2/s) observed at 6 dS/m salinity level in Padamdungari population. The net photosynthetic rate decreased when salinity level increased as well as age increased in Teak (Table 2).

Table 2: Interaction effect of population and salinity level of irrigation water on net photosynthetic rate (P_n) in five Teak populations grown at five levels of saline water upto 120 days

Population x Salinity level	P_n (umol/m^2/s			
	30 days	60 days	90 days	120 days
Bhenskatri				
0.2 dS/m	10.12	14.64	11.75	4.89
1 dS/m	10.39	11.47	9.35	3.98
2 dS/m	12.17	5.86	6.08	7.32
4 dS/m	9.45	3.84	4.09	4.80
6 dS/m	8.42	1.60	2.48	2.00
Junagadh				
0.2 dS/m	11.67	10.50	14.22	11.37
1 dS/m	8.86	6.30	8.50	6.80
2 dS/m	6.88	4.28	6.90	5.52
4 dS/m	4.76	3.08	5.20	4.16
6 dS/m	2.91	1.50	2.35	1.88
Padamdungari				
0.2 dS/m	10.17	11.02	10.08	8.06
1 dS/m	8.38	4.97	9.05	7.24
2 dS/m	6.97	1.75	2.51	1.66
4 dS/m	4.25	1.25	0.89	0.42
6 dS/m	3.36	0.33	0.32	0.25
Subir				
0.2 dS/m	6.11	8.77	3.88	3.10
1 dS/m	4.98	6.85	2.58	2.06
2 dS/m	3.14	5.59	1.75	1.40
4 dS/m	1.17	3.85	0.91	0.73
6 dS/m	0.45	2.65	0.48	0.38
Waghai				
0.2 dS/m	9.51	11.13	5.37	4.30
1 dS/m	6.82	8.73	2.96	2.37
2 dS/m	4.74	7.06	1.46	1.17
4 dS/m	4.01	5.65	0.78	0.62
6 dS/m	3.18	3.41	0.46	0.37
$LSD_{0.05}$				
Population x salinity level	1.30	1.50	1.14	0.87

Effect of salinity levels and population source on transpiration rate of Teak

In the present research study, the transpiration rate varied significantly under the influence of salinity stress. The analysis of variance showed significant effect of salinity levels and populations for transpiration rate trait. At the end of experiment, the maximum transpiration (1.39 mmol/m^2/s) observed at 0.2 dS/m whereas, the minimum transpiration rate (0.53 mmol/m^2/s) observed at 6 dS/m. The transpiration rate decreased when salinity level increased. The population also showed significant variation for salinity tolerance in Teak. The maximum transpiration rate found in Junagadh population (1.39 mmol/m^2/s) while, the transpiration rate observed in Waghai population (0.57 mmol/m^2/s) in Teak (Table 3).

In the present research study, the transpiration rate varied significantly under the interaction effect of population and levels of salinity. At the end of experiment, the maximum transpiration rate (2.02 mmol/m^2/s) observed at 0.2 dS/m control in Padamdungari population whereas, the minimum transpiration rate (0.16 mmol/m^2/s) observed at 6 dS/m salinity level in Padamdungari population. The transpiration rate decreased when salinity level increased in Teak (Table 4).

Table 3: Main effects of population and salinity level of irrigation water on transpiration rate (E) in five Teak populations grown at five levels of saline water upto 120 days

Treatments	E (mmol/m²/s)			
	30 days	60 days	90 days	120 days
Population				
Bhenskatri	1.45	1.62	1.46	1.28
Junagadh	1.00	0.68	1.73	1.39
Padamdungari	1.23	0.80	1.37	0.89
Subir	1.06	1.40	0.87	0.70
Waghai	1.25	1.11	0.71	0.57
Salinity level				
0.2 dS/m	1.49	1.68	1.81	1.39
1 dS/m	1.18	1.27	1.53	1.15
2 dS/m	1.18	1.07	1.20	1.03
4 dS/m	1.09	0.92	0.88	0.72
6 dS/m	1.05	0.68	0.74	0.53
$LSD_{0.05}$				
Population	0.17	0.17	0.19	0.17
Salinity level	0.17	0.17	0.19	0.17

Table 4: Interaction effects of population and salinity level of irrigation water on transpiration rate (E) in five Teak populations grown at five levels of saline water upto 120 days

Population x Salinity level	E (mmol/m²/s)			
	30 days	60 days	90 days	120 days
Bhenskatri				
0.2 dS/m	1.37	2.25	1.60	1.01
1 dS/m	1.19	2.36	1.75	1.01
2 dS/m	1.62	1.53	1.49	1.91
4 dS/m	1.47	1.24	1.40	1.55
6 dS/m	1.63	0.74	1.09	0.92
Junagadh				
0.2 dS/m	1.86	0.87	2.12	1.70
1 dS/m	0.76	0.51	1.99	1.59
2 dS/m	0.89	0.63	2.27	1.82
4 dS/m	0.75	0.72	1.37	1.10
6 dS/m	0.72	0.64	0.92	0.74
Padamdungari				
0.2 dS/m	1.56	1.93	2.53	2.02
1 dS/m	1.40	0.77	2.12	1.69
2 dS/m	1.16	0.66	0.92	0.40
4 dS/m	1.12	0.50	0.64	0.16
6 dS/m	0.90	0.16	0.67	0.16
Subir				
0.2 dS/m	1.26	1.92	1.51	1.22
1 dS/m	1.26	1.61	0.95	0.76
2 dS/m	1.15	1.28	0.75	0.60
4 dS/m	0.80	1.26	0.53	0.42
6 dS/m	0.82	0.91	0.62	0.49
Waghai				
0.2 dS/m	1.38	1.43	1.29	1.03
1 dS/m	1.30	1.08	0.84	0.67
2 dS/m	1.10	1.23	0.55	0.44
4 dS/m	1.30	0.86	0.46	0.37
6 dS/m	1.19	0.95	0.41	0.33
$LSD_{0.05}$				
Population x salinity level	0.37	0.38	0.42	0.37

Effect of salinity levels and population source on stomatal conductance of Teak

In the present research study, the stomatal conductance varied significantly under the influence of salinity stress. The analysis of variance showed significant effect of salinity levels and populations for stomatal conductance trait. At the end of experiment, the maximum stomatal conductance (164.73 mmol/m²/s) observed at 0.2 dS/m whereas, the m stomatal conductance (100.38 mmol/m²/s) observed at 6 dS/m.

Table 5: Effects of population and salinity level of irrigation water on stomatal conductance (g_s) in five Teak populations grown at five levels of saline water upto 120 days

Treatments	g_s (mmol/m²/s)			
	30 days	60 days	90 days	120 days
Population				
Bhenskatri	365.60	239.65	342.84	196.01
Junagadh	284.15	145.29	276.68	168.20
Padamdungari	311.51	160.05	189.85	154.40
Subir	167.04	283.10	142.75	113.17
Waghai	319.15	220.42	130.30	93.99
Salinity level				
0.2 dS/m	282.89	289.55	245.26	164.73
1 dS/m	356.93	254.51	281.08	158.54
2 dS/m	298.35	205.14	248.25	181.76
4 dS/m	261.85	163.52	170.34	120.36
6 dS/m	247.45	135.78	137.48	100.38
$LSD_{0.05}$				
Population	42.48	34.25	28.72	21.23
Salinity level	42.48	34.25	28.72	21.23

Table 6: Interaction effects of population and salinity level of irrigation water on stomatal conductance (g_s) in five Teak populations grown at five levels of saline water upto 120 days

Population x Salinity level	g_s (mmol/m²/s)			
	30 days	3-60 days	90 days	120 days
Bhenskatri				
0.2 dS/m	255.31	351.62	322.54	123.32
1 dS/m	346.12	353.00	341.97	102.19
2 dS/m	348.26	212.93	443.75	360.57
4 dS/m	410.60	158.78	349.19	237.25
6 dS/m	467.72	121.92	256.76	156.70
Junagadh				
0.2 dS/m	366.96	184.46	260.52	145.42
1 dS/m	308.74	143.73	325.82	197.04
2 dS/m	325.86	118.62	415.74	235.02
4 dS/m	226.30	149.79	212.85	134.84
6 dS/m	192.90	129.85	168.47	128.69
Padamdungari				
0.2 dS/m	278.05	318.65	225.65	285.68
1 dS/m	485.96	165.81	395.27	242.09
2 dS/m	327.11	139.38	137.57	114.62
4 dS/m	264.58	111.57	100.89	66.70
6 dS/m	201.86	64.83	89.85	62.91
Subir				
0.2 dS/m	219.94	395.86	210.89	158.42
1 dS/m	192.04	361.66	176.43	133.65
2 dS/m	195.31	261.52	135.97	108.27
4 dS/m	124.02	202.04	98.33	84.15
6 dS/m	103.90	194.42	92.13	81.37
Waghai				
0.2 dS/m	294.20	197.16	206.72	110.83
1 dS/m	451.78	248.38	165.91	117.71
2 dS/m	295.19	293.25	108.20	90.30
4 dS/m	283.72	195.42	90.45	78.88
6 dS/m	270.84	167.87	80.21	72.24
$LSD_{0.05}$				
Population x salinity level	94.98	76.57	64.23	47.47

The stomatal conductance decreased when salinity level increased. The population also showed significant variation for salinity tolerance in Teak. The maximum stomatal conductance found in Bhenskatri population (196.01 mmol/m²/s) while, the stomatal conductance observed in Waghai population (93.99 mmol/m^2/s) in Teak (Table 5).

In the present research study, the stomatal conductance varied significantly under the interaction effect of population and levels of salinity. At the end of experiment, the maximum stomatal conductance (360.57 mmol/m^2/s) observed at 4 dS/m salinity level in Bhenskatri population whereas, the minimum stomatal conductance (62.91 mmol/m^2/s) observed at 6 dS/m salinity level in Padamdungari population (Table 6).

Thus, gas exchange physiological parameters significantly varied with salinity level from population to population during research study period. It is evident from these table that the net photosynthetic rate (Pn, µmol), transpiration rate (E) and stomatal conductance (g_s) significantly decreased at higher concentration of salt. Though these parameters were less affected in Junagadh and Bhenskatri populations of teak.

Discussion

In present study, five populations were evaluated for physiological parameters under five different levels of saline irrigation regime. There was large variation recorded for physiological characters among all the population and salinity levels and their interactions. Though, all the five populations of Teak survived under continuous supply of saline water and the detrimental effect of salinity was evident at higher dose (EC ≥ 4 dS/m). The plant growth is controlled by a multitude of physiological, biochemical, metabolic and molecular processes, where photosynthesis in green plants is a key phenomenon which contributes substantially to the plant growth and development. The chemical energy consumed in a number of metabolic processes in plants is derived from the process of photosynthesis in plants which is capable of converting sun light energy into an operational chemical form of energy. This key process of photosynthesis occurs in all green plants even occurring in oceans as well as in photosynthetic bacteria (Taiz and Zeiger 2010) [19].

However, stressful environment conditions including salinity, drought, and unfavourable temperatures, and other locality factors considerably hamper the process of photosynthesis in most plants by altering the ultrastructure of the organelles and concentration of various pigments and metabolites including protein, enzymes involved in this process as well as stomatal regulation. In present research study, the photosynthesis reduced at higher salinity levels. The reduction further increased as time of salinity increased. Photosynthetic pigments present in the photosystems are believed to be damaged by such stress factors resulting in a reduced light-absorbing capacity of both photosystems (PSI and PSII) and hence a reduced photosynthetic efficacy (Zhang *et al.* 2011; Geissler *et al*. 2009) [20, 9].

In teak, the reduced stomatal conductance observed along with transpiration and photosynthesis rate. However, there was significant variation for reduction in physiological activity among all the populations. The tolerant population yielding higher biomass comparatively showed lower decrease. The regulation of leaf stomatal conductance (g_s) is a key phenomenon in plants as it is vital for both a prevention of desiccation and CO_2 acquisition (Dodd 2003, Medici *et al*. 2007) [7, 14]. Stomata closure in response to drought and salinity stress generally occurs due to decreased leaf turgor and atmospheric vapor pressure along with root-generated chemical signals (Chaves *et al*. 2009) [3]. Thus, the decrease in photosynthetic rate under stressful conditions (salinity, drought, and temperature) is normally attributed to a suppression in the mesophyll conductance and the stomata closure at moderate and severe stress (Flexas *et al*. 2004, Chaves *et al*. 2009) [3, 8]. The effects of salinity and drought on photosynthesis are attributed directly to the stomatal limitations for diffusion of gases, which ultimately alters photosynthesis and the mesophyll metabolism (Parida *et al*. 2005, Chaves *et al*. 2009) [18, 3]. Thus, all these studies corroborate the present result of the experiment. Such variations should be effectively captured for further tree breeding and tree improvement programme to cope up with increasing salinization problems worldwide.

Conclusion

From the present investigation, it can be concluded that the physiological parameters of Teak were reduced when the salt concentration increased in the early stage. Junagadh and Bhenskatri population were performed better among all the five populations at all five salinity levels. However, all five populations of teak survived under continuous supply of saline water and the detrimental effect of salt was evident at higher dose (EC ≥ 4 dS/m). Thus, Junagadh and Bhenskatri population was recommended for saline areas for plantation purposes. Population variability should be effectively captured for further tree improvement of teak for salinity resistance.

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Competing interests

Authors have declared that no competing interests exist.

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