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Assessment of Clonal differences in Poplar *(Populus deltoides)* by Virtual Microscopy using Foldscope

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ABSTRACT: *Populus deltoids* is a commercially important, dioecious tree species grown widely in the Kashmir valley. Female plants produce huge amounts of cottony seed that pose respiratory problems. Morphologically, females are indistinguishable from the male plants until the plants attain sexual maturity in 5-8 years. Molecular technology can help detect the sex of genotypes in the initial stages, but these technologies require not only resources but are also not affordable in resource-limited environments. In the present study, we tried to find out differences in stomatal density among male and female clones of *P. deltoids* with the help of a foldscope. Five male and five female clones of the species were raised in a replicated trial. The average stomatal density in the mature leaves of one year old seedlings was found 379.98mm^{-2}. There was no statistically significant difference in the stomatal density in upper and lower leaf surfaces. However, the stomatal density on the lower leaf surface in female clones was significantly higher (196.86 mm^{-2}) than that in male clones (183.12 mm^{-2}). The clones differed in growth characters. But this difference was not correlated to the difference in stomatal density.

Keywords: *Populus deltoides*, stomatal density, foldscope, male clones, female clones.

INTRODUCTION

Poplars (*Populus deltoides*) are widely grown in plantations for various industrial products like pulp, paper, veneer, excelsior, engineered wood products, lumber, and energy. In Kashmir valley, J&K, poplars are grown on a large scale as raw material for the plywood industry.

Poplars exhibit sexual dimorphism i.e., male and female flowers are borne on separate individuals. However, the plants are not distinguishable until they attain sexual maturity (about 5-8 years). This longer gestation interval prolongs the gestation period in its breeding and frustrates poplar breeders and farmers as they cannot identify which trees they should mate or plant until sexual maturity. The problem is further compounded as forestry - unlike agriculture - is a long-term proposition and mistakes committed once are reflected after several years (Kumar, 2017).

In the state of Jammu and Kashmir, ascertaining the sex of the plant at the seedling stage has acquired a new dimension. Female plants produce huge amounts of undesirable cottony seed that is undesirable especially when grown near habitation. This cottony seed floats in the air and has been widely accepted as a major cause of respiratory problems/ discomfort among the common people. Given this health hazard, the J&K high court has ordered the removal of all the female poplar plants growing near the populace and restricted to male clones for all new plantations.

Molecular techniques can help in the early detection of sex, but these technologies are not only resource demanding and unaffordable in a resource-constrained environment, but also need higher degree skills. There are some reports, however, of sex-associated differences in morphology and physiology in poplars. Leaves of male and female trees of *P. ciliata* are differentiated on the basis of three qualitative characters such as the shape of the base of leaf blade, sinus with petiole, and leaf margin (Kumari *et al.,* 2016). Clonal differences have been reported by Broekhulzen (1964) by developing a key based on leaf characters for the identification of one year old poplar plants. Grant & Mitton (1979) found female ramets had higher mean annual incremental growth than male ramets.

With these facts and findings in view, this study was conducted to find differences in stomata count in male and female clones of *Populus deltoids* using a foldscope. Foldscope is the ultra-affordable paper microscope. It was designed to be portable and durable, while performing on par with conventional research microscopes (140X magnification and 2-micron resolution). can be that can be assembled from a punched sheet of cardstock, a spherical glass lens, a light-emitting diode, and a diffuser panel, along with a watch battery that powers the LED (Grant & Mitton,

1979). Once assembled, the Foldscope is about the size of a bookmark. It was developed by Manu Prakash. It is part of the "frugal science" movement which aims to make cheap and easy tools available for scientific use in the developing world.

MATERIALS AND METHODS

A. Plant materials

We procured five male and five female clones of *Populus deltoids* from WIMCO seedlings, ITC, Rudrapur and raised them in the nursery at the Faculty of Forestry, Benhama, Ganderbal, Kashmir. The rooted cuttings were procured in April, 2018 and immediately planted in the nursery.

B. Experimental layout

The experiment was laid out in an RCBD with 4 replications. The number of seedlings per plot was 20. Each replication consisted of 10 different clones. Thus, the total number of seedlings raised in each clone was 80 in all four replications.

C. Growth data

The observations were recorded at the end of the growing season, in December, 2018. Plant height and collar diameter were using a measuring tape.

D. Glass slide preparation

Imprints of epidermal cells and stomata were taken from fully expanded, mature *P. deltoides* leaves formed from current year meristems, initiated and developed during the growing season. The imprints were made by coating a 25mm^2 area on leaf surface with clear nail varnish, covering with 'cellotape', applying pressure, and replacing onto a glass microscope slide following (Tricker *et al.*, 2005).

E. Foldscopic observations

We observed the stomata of poplar leaves in the foldscope and counted their number in a given area with the help of an ocular stage micrometer. Slides of stomata were prepared on a micrometer glass slide with an engraved scale having the least count of 0.01 mm so that the stomata were superimposed over the scales of the micrometer. We took the photographs of the slides using a mobile camera (having 12 mp resolution) coupled with the foldscope as shown in Figure 1. The photographs were then transferred to the personal computer for further processing, stomata counting and area calculations as shown in Figure 2.Stomata on upper and lower leaf surfaces were counted separately and these were added together to yield total stomatal density for each clone.

F. Statistical analysis

The data was subjected to ANOVA with the post hoc comparison values calculated for plant height and collar diameter to find out differences among the poplar clones. The paired t-test was used to find any significant differences with respect to stomata count on the lower and upper leaf surface of the clones. Student's t-test was used to find out differences between the male and female poplar clones based on their average stomata count. The correlation analysis was used to find the strength of the relation between stomatal density with plant height and collar diameter. The data were analyzed using statistical packages R studio (Version 1.4.2.) and SPSS.

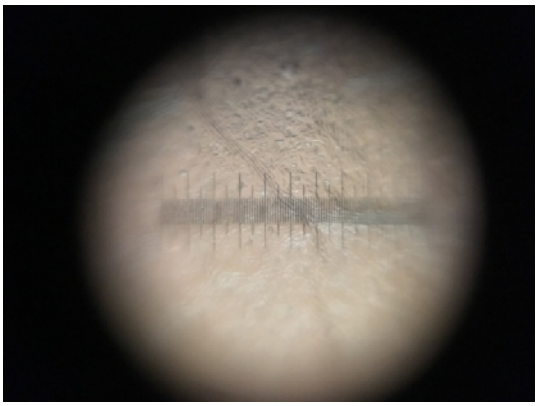

Fig. 1. Original photograph (taken by mobile attached to the foldscope) of the stomata superimposed on micrometer slide.

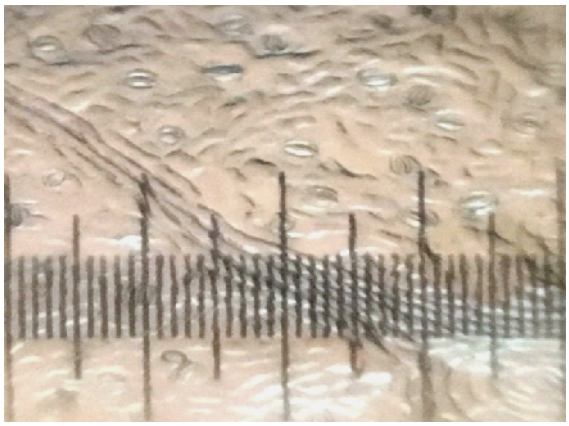

Fig. 2. The processed enlarged image of the same photograph for stomatal counting.

RESULTS AND DISCUSSION

The result of the study revealed that the average stoma on the leaf surface was 47.73 in 0.1256 mm^2 of leaf area as shown in Table 1. This comes out to be 379.98 stomata in one square millimeter. The stomatal density varied significantly amongst the clones with the highest value in clone G48 (53.92 in 0.1256 mm^2) and lowest (43.25 in 0.1256 mm^2) in Udai.

We also tried to find out whether the lower surface had a higher stomatal density than the upper leaf surface. The result shows that the lower surface had slightly higher stomatal density (24.26 in 0.1256 mm^2) than the upper surface (23.47 in 0.1256 mm^2), though the difference was statistically non-significant as shown in Table 2. There was also a non-significant difference amongst the clones with respect to upper leaf stomatal density. However, the clonal difference in the stomatal density on the lower leaf surface was significant.

Table 1: Mean (±S.E.) stomatal density of *Populus deltoids* clones.

Clones	Mean (Number In 0.1256 Mm^2)	*Number Per Mm^2
Udai	43.25±1.38	344.35
W81	47.50±1.39	378.18
A26	46.84±2.42	372.88
W39	48.17±0.99	383.49
W109	50.50±3.51	402.07
G48	53.92±1.83	429.27
108	47.42±2.85	377.52
W83	46.08±2.87	366.91
W22	45.00±1.11	358.28
W110	48.58±0.83	386.81
Mean	**47.73**±1.87	**379.98**
CD = 5.452; SE(d) = 2.643; CV = 7.832		

* Converted values

Table 2: Mean (±S.E.) stomatal density on upper and lower leaf surfaces in *Populus deltoids.*

Stomata / Clones	Upper		Lower	
	Number in 0.1256 mm^2	*Number per mm^2	Number in 0.1256 mm^2	*Number per mm^2
UDAI	22.50±0.799	179.14	20.75±1.092	165.21
W81	23.75±1.049	189.09	23.75±0.551	189.09
A26	22.83±1.126	181.79	24.00±1.569	191.08
W39	23.75±0.906	189.09	24.42±0.975	194.40
W109	25.33±1.683	201.70	25.17±1.898	200.37
G48	29.17±1.613	232.22	24.75±0.672	197.05
W108	24.08±1.436	191.75	23.33±1.737	185.77
W83	23.50±1.686	187.10	22.58±1.423	179.80
W22	23.25±0.567	185.11	21.75±1.075	173.17
W110	24.42±0.975	194.40	24.17±0.776	192.41
Mean	**24.26±1.145**	**193.14**	**23.47±1.105**	**186.84**
	CD = 3.341; SE(d) = 1.620; CV = 9.443		N.S.	

* Converted values

Pointeau & Guy (2014) found significant variation in stomatal count in two poplar species, *P. trichocarpa* and *P. balsamifera*. They also found that stomatal density was higher in *P. balsamifera* than in *P. trichocarpa.* Their study revealed that *P. balsamifera* had almost no stomata on the upper leaf surface. Stomatal densities found in *P. deltoides* in the present study were higher than these values. However, stomatal densities in *P. deltoides*, as reported by Brazen *et al.*, (1992) and Drew & Bazzaz (1979) are quite comparable with the values found in the present study. Their studies also revealed that differences in stomatal count on upper and lower stomata were non-significant as we found also in the present study.

For comparing male and female clones in terms of the stomatal density we grouped the clones in two groups of five male and five female clones as shown in Table 3. We found that the two groups were statistically different from each other (p = 0.050). The stomatal density of the female clones (24.72 in 0.1256 mm^2) was more than those of male clones (23.00 in 0.1256 mm^2). Analysis of growth data - seedling height and collar diameter - revealed significant differences among the clones (Table 4). The plant height of six clones was more than the mean seedling height (99.11 cm). Clone W81 performed (116.77 cm) better than all other clones.

Table 3: Stomatal density on the lower leaf surface in male and female clones of *Populus deltoides.*

Sr. No.	Male clones	Mean (number in 0.1256 mm^2)	Mean (number per mm^2)	Female clones	Mean (number in 0.1256 mm^2)	Mean (number per mm^2)
1.	Udai	21.63	172.17	W39	24.08	191.75
2.	W81	23.75	189.09	W109	25.25	201.04
3.	A26	23.42	186.44	G48	26.96	214.64
4.	W108	23.71	188.76	W83	23.04	183.45
5.	W22	22.50	179.14	W110	24.29	193.40
	Mean	**23.00**	**183.12**	**Mean**	**24.72**	**196.86**
t-value = -2.215; p-value = 0.050						

Similarly, six clones had collar diameters greater than the overall mean collar diameter (10.84 cm) and clone W81 was the best performing clone (13.46 cm). We also explored the possible relationship between stomatal density and growth data. However, the correlation coefficients were statistically non-significant. It implies that higher stomatal density does not translate into higher growth rates. Stomatal conductance and not stomatal density decide the exchange of gases across leaf surface and hence growth rate (Tricker *et al.*, 2005).

Table 4: Seedling height and collar diameter of one-year-old seedlings of *Populus deltoides*.

Clones	Plant height (cm) Mean (±S.E.)	Collar diameter (cm) Mean (±S.E.)
UDAI	100.55±6.36	9.685±1.330
W81	116.77±8.42	13.465±1.301
A26	85.66±7.17	8.058±1.136
W39	111.69±30.02	11.408±3.124
W109	102.59±22.73	11.455±2.465
G48	82.88±23.67	8.800±2.148
108	101.96±27.80	10.613±2.459
W83	89.65±18.48	11.560±3.129
W22	101.50±21.58	10.295±2.401
W110	97.82±6.58	13.045±2.489
Mean±S.E.	**99.11±12.347**	**10.84±1.279**
CD	36.52	3.49
SE(d)	17.462	1.809
CV	24.918	23.606

CONCLUSION

The findings of the study reveal that foldscope can be used to estimate stomatal density in trees. We also found a significant difference in stomatal density between male and female clones of *Populus deltoides*. Thus the technique can be used to develop an easy, field-based tool to distinguish female clones from male clones in the nursery stage well before the plants attain sexual maturity.

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