

Improving Growth Performance of *Jatropha curcas* by Inducing Polyploidy through Colchicine Treatment

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Abstract

This study was planned to assess genetic variation in *Jatropha curcas* as affected by colchicine treatments. During the experiment, seeds of *Jatropha curcas* were collected from mature and healthy *Jatropha* plants and were soaked in different concentrations of colchicines. The second treatment was that of time of soaking (seeds were soaked in different concentrations for different time durations). The results clearly revealed that by increasing the concentration of colchicine treatment, parameters like stomatal density, and size of the guard cells were reduced.

Keywords

Jatropha, Polyploidy, Colchicine, Biofuel

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1. Introduction

The ever worsening energy crisis across the globe has triggered large scale research projects to identify economical, eco-friendly and sustainable sources of alternative fuel [1]. Of those numerous alternate options as suggested by scientists, biofuel is a promising source to combat the Damocles' sword of energy crisis. *Jatropha curcas*, one of the most productive and widely experimented biofuel tree plants has been known for its high oil content of seed and its potential to grow on marginal lands with lesser requirements of water and fertilizer [2]. The oil extracted from the seeds of this energy plant can be utilized for running low power engines and has been used to power different agricultural machinery [3].

Although, *Jatropha curcas* seeds are pretty rich in oil content, it has shown low productivity of 625 - 1875 kg·ha⁻¹·yr⁻¹ and lesser physical vigor [4]. It is therefore essential to carry out studies to figure out a suitable yield-increasing strategy for *Jatropha* plant. Since this tree plant has only limited genetic diversity, it becomes difficult to address the above mentioned issue through conventional breeding programs [5]. An alternate way to encounter this problem, as has been experimented by various researchers, is to induce mutation through seeds of *Jatropha* plant [6] to develop a new variety that may possibly give higher per hectare yield. Research studies have reported that application of colchicine increases the chromosome number and thus leads to bigger seed and flower sizes. As suggested by [7], colchicine doubled the number of chromosomes in watermelon, thus leading to bigger seeds. [8] reported similar results for ginger plant when treated with colchicine.

Since Pakistan is one of those countries where climatic conditions largely support the growth of *Jatropha* crop, the current research therefore involves the use of colchicine to induce tetraploidy in *Jatropha* so as to develop a new variety that would possibly produce greater yield.

2. Materials and Methods

Seeds of *Jatropha curcas* were taken from 2 years old mature *Jatropha* plants. In order to mutate the seeds, different concentrations of colchicine were taken. Besides, seeds were soaked in those concentrations for different number of hours (6, 12, 24, 48). The colchicines concentrations were 0.25, 1.25 and 2.5 mM and the set of seeds untreated with colchicine was taken as control in this experiment. Completely Randomized Design was used in this experiment in which there were three replications for each treatment while 10 seeds of *Jatropha curcas* were taken in each replication.

The treated seeds were grown in green house conditions. The experiment was conducted from March 2014 to May 2015 (90 days). At the end of the experiment, plant characteristics like stomatal density and the size (length and width) of guard cells were determined.

3. Statistical Analysis and Experimental Design

The experiment was framed using completely randomized design (CRD) ANOVA (Analysis of variance) was carried out and mean values of treatments were compared using DMRT (Duncan's multiple range test).

4. Results and Discussion

As depicted in **Table 1**, stomatal density of *Jatropha curcas* does not seem to have been significantly affected by the colchicines treatments. Although the stomatal density has increased with increasing the colchicines concentration, the highest stomatal density was achieved with treatment T11 (1.25 mM, 24 hours of soaking). Stomatal density is an important parameter while considering the growth performance of a particular plant species. Since stomata is the gateway of gaseous exchange for plants, higher density of stomata leads to more vigorous growth of plants [9].

Length of the guard cells was significantly affected by the application of colchicine. Length of the guard cells seems to have been minimum at lower and highest concentrations of colchicine. At moderate concentrations, maximum value was obtained for this parameter. Highest value for the length of the guard cells was obtained at treatment T6 (0.25 mM, 12 hours of soaking). Similarly, the width of the guard cell had also shown significant response to the application of colchicine. The induction of tetraploidy brought significant difference to the plant growth by affecting the width and the length of the guard cells. The width of the guard cells was recorded maximum in treatment T3 (0 mM, 24 hour). Although there was no trend observed in the reading, yet an increase in the concentration and soaking duration seemed to have negatively affected the width of the guard cells

Table 1. Effect of colchicine application on various growth parameters of *Jatropha curcas*.

Treatment	Soaking Concentration	Soaking Duration	Stomatal Density	Length of Guard Cell	Width of Guard Cell
T1 (Control)	0 mM	6 Hours	9.12 ± 1.2	0.035 ± 0.00012 ab	0.025 ± 0 c
T2	0 mM	12 Hours	8.91 ± 0.81	0.038 ± 0.00001 ab	0.04 ± 0 ab
T3	0 mM	24 Hours	9.21 ± 00	0.041 ± 0.00032 ab	0.044 ± 0.0004 a
T4	0 mM	48 Hours	9.81 ± 0.23	0.04 ± 0.0014 ab	0.045 ± 0.0003 a
T5	0.25 mM	6 Hours	10.59 ± 0.13	0.037 ± 0.00014 ab	0.039 ± 0.0004 b
T6	0.25 mM	12 Hours	10.98 ± 0.98	0.044 ± 0.00019 a	0.04 ± 0 ab
T7	0.25 mM	24 Hours	11.24 ± 1.1	0.036 ± 0.00018 ab	0.036 ± 0.0011 ab
T8	0.25 mM	48 Hours	11.12 ± 0.35	0.036 ± 0.00051 ab	0.037 ± 0 ab
T9	1.25 mM	6 Hours	13 ± 1.2	0.034 ± 0.00006 ab	0.035 ± 0.0001 ab
T10	1.25 mM	12 Hours	12.85 ± 1.23	0.039 ± 0.00019 b	0.034 ± 0.0003 ab
T11	1.25 mM	24 Hours	13.1 ± 0.94	0.034 ± 0.00051 ab	0.034 ± 0 ab
T12	1.25 mM	48 Hours	12.21 ± 0.99	0.034 ± 0.00017 ab	0.035 ± 0.0003 ab
T13	2.5 mM	6 Hours	12.23 ± 0.31	0.036 ± 0.0003 ab	0.038 ± 0.0012 b
T14	2.5 mM	12 Hours	12.48 ± 1.51	0.04 ± 0.00041 b	0.036 ± 0 ab
T15	2.5 mM	24 Hours	12.1 ± 1.00	0.034 ± 0.0003 ab	0.037 ± 0.0001 b
T16	2.5 mM	48 Hours	12.34 ± 0.87	0.038 ± 0.00021 b	0.04 ± 0 a

in *Jatropha* plants. As suggested by [10], the significant difference in width and length of guard cells was possibly due to the fact that most of the normal seeds were diploid (colchicine was not applied). However, when different concentrations of colchicine were applied, it would have resulted in different types of mixploidy. Induction of tetraploidy, as would be expected by application of colchicine treatments, would have caused difference in the size of guard cells among the mutant seed plants.

This research study has highlighted the possible outcomes of application of colchichone on *Jatropha* seeds through seed soaking. The study showed that the application of colchicine brought changes in the ploidy level and the results were reflected in terms of variations in the value of width and length of guard cells on mutated *Jatropha* plant leaves. The growth parameters of the tetraploid plant were however lower as compared to the normal normal *Jatropha* plants which indicated that the subsequent yield would also be lower as compared to the normal *Jatropha* plants.

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