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Alteration of Gene Expression by Smoke (*Cynodon dactylon (L.) Pers*) Treated Water in Tomato Seed Germination

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Abstract

Smoke released from burning vegetation contains a chemical constituent that influences germination of crop seeds. The application of smoke treated water has been investigated in horticultural crop seeds around the world. In present investigation, the smoke treated water has shown an inhibitory effect on tomato seeds by delaying the germination, also shown low percentage of seed germination & seedling vigor. The seeds were tested for their germination percentage by using the moist petri plate method. The study of effect of smoke treated water at protein expression level has been carried out with SDS-PAGE. The molecular weight of inhibitory protein which supposed to be responsible for the delay in germination has been identified by using BSA as molecular marker.

Keywords: Gene expression; Smoke treated water; *Cynodon dactylon (L.) Pers*; Tomato; Seed germination.

INTRODUCTION

There are several factors affecting seed germination like seed viability, optimal moisture, proper temperature, light, and ample oxygen etc., Seed germination includes three phases according to physiological processes namely, Activation, Digestion & Translocation and Seedling Growth. In Andhra Pradesh, Madanapalle which is located in Chittoor district is well known as “Ooty of Andhra” is occupying the first position in tomato cultivation both area wise and production wise. Tomatoes, aside from being tasty, are very healthy as they are a good source of vitamins A, Vitamin C and Lycopene, an antioxidant. Lycopene has also been shown to protect against oxidative damage in many epidemiological and experimental studies. In addition to its antioxidant activity, other metabolic effects of lycopene have also been demonstrated. The richest source of lycopene in the diet is tomato and tomato derived products [1]. Tomato consumption has been associated with decreased risk of breast cancer [2], head and neck cancers [3] and might be strongly protective against neurodegenerative diseases [4,5,6]. Tomatoes, tomato sauces and puree are said to help lower urinary tract symptoms (BPH) and may have anticancer properties [7]. Tomato consumption might be beneficial for reducing cardiovascular risk associated with type 2 diabetes [8].

Smoke released from burning vegetation contains a chemical signal that triggers germination of both fire climax and non-fire climax species from different parts of the world. It is used in horticulture to stimulate seed germination of wildflower species and can break dormancy and improve germination of vegetable crops, such as lettuce and celery. Smoke can be applied to seed immediately before sowing, or the seed may be pretreated and stored until conditions are appropriate for sowing. Both smoke and aqueous smoke -water are active in this respect. A very clear concentration effect, resembling that of hormonal responses has been established with aqueous smoke solutions. Smoke extracts interact with gibberellins, cytokinins, abscisic acid and ethylene in photoblastic and in thermodormant seed. Smoke may well be the overriding trigger for germination in relation to specific growth habits, regeneration strategies, seed storage, seed sizes, dispersal modes and structures for a large number of species growing in fire-prone habitats. In many species, the effects of smoke are astounding. Smoke, for example, has been reported to enhance the germination of the South African plants *Erica clavisepala* and *Restio festuciformis* by more than 7,000% and 25,000%, respectively. Seeds treated with smoke retain an enhanced ability to germinate even after 1 year of storage [9]. The finding that the combustion of cellulose alone has a stimulatory effect on germination has raised the possibility that one of the bioactive components of plant-derived smoke may originate from a thermal breakdown product of hemicellulose or cellulose [10]. Aqueous smoke extracts prepared from a range of plants, and extracts prepared by heating agar and cellulose, contained compounds that stimulated the germination of light-sensitive lettuce (*Latuca sativa*) seeds. Chromatographic evidence suggested that the same active compound(s) is produced from *T. triandra* leaves, agar, and cellulose. They identified 1,8-cineole as an active germination enhancer in smoke. Different conclusions have been reached about a possible role for octanoic acid being an active factor in smoke. Smoke, on the other hand will not stimulate the seed germination. Aqueous extracts from leaf sources of *Eucalyptus* species and *Azadirachta* species were shown the inhibitory effect on the seed germination as well as seedling vigor [11].

MATERIALS AND METHODS

Sources of Smoke Treated water & Tomato Seeds

Smoke water is collected from burning grass (*Cynodon dactylon (L.) Pers*) collected from Madanapalle Institute of Technology and Science campus, Madanapalle, Chittoor

(D), Andhra Pradesh, INDIA. Tomato seeds of Vigro brand, Golden seeds of variety F1 Vaishnavi (2082), LOT-8111004, Germination % of 70% and purity 98% are used in present investigation. The different kinds of tests were carried to find out dormancy breaking, germination and gene expression studies. The experiments conducted with *Cynodon dactylon (L.) Pers* smoke water and distilled water were as follows a) Seed Germination Test b) Seedling Vigor Test c) Identification of proteins.

Seed germination Test

This test aims to get the viability of the seeds. To prove the viability, percentage of seed germination tests were conducted by taking 200 seeds for the experiments. The care was taken and the unhealthy seeds were removed by physical verification. The healthy seeds were used for seed germination test. The results of seed germination test have obtained by using the formula:

$$PGS = \frac{TNGS}{TNHS} \times 100$$

Where,
PGS- Percentage of seed Germination
TNGS- Total number of germinated seeds
TNHS- Total number of healthy seeds

Seedling vigor Test

Vigor tests aim to measure the ability of the seed to perform well under unfavorable conditions and are used to discriminate between seeds for storage and to discriminate between seeds of maximal performance. Hence we can consider that seedling vigor test is the closest measure for potential field performance. Seedling vigor can be calculated by using the formula:

Seedling vigor
= (Shoot length + Root length) x Germination percentage.

Identification of proteins

This was done by using SDS-Poly Acrylamide Gel Electrophoresis. Electrophoresis is the study of the movement of charged particles in an applied electric field. Any charged ion or molecule migrates when placed in an electric field. The rate of migration of a compound depends on its net charge, size, shape and the applied current. This can be represented by the following equation

$V = E \times q/f$

V- Velocity of migration of the molecule
E - Electric field in volts/cm
q - Net electrical charge
f - The frictional coefficient of the molecule

The gel was collected and by observing the distances moved by protein bands and the tracking dye, the Rm values of the seed proteins were estimated using the formula,

$$\mathbf{Rm} = \frac{\text{(Distance between origin and band)}}{\text{(Distance between origin and tracking dye)}}$$

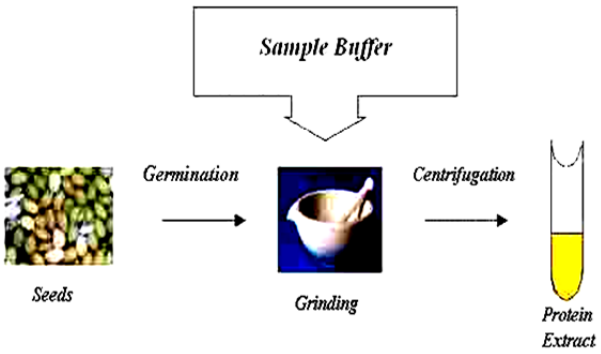

Fig. 1 Procedure for Sample Preparation

RESULTS AND DISCUSSION

The percentages of germination in the case of distilled water treated seeds (control) are 89%, *Cynodon dactylon (L.) Pers* smoke water treated water is 56%. The percentage of germination is maximum in case of distilled water treated seeds and least in the case of *Cynodon dactylon (L.) Pers* smoke water treated seeds (**Fig.2**).

The seedling vigor of distilled water treated seeds is 196.245, whereas smoke water treated seeds is 6.16. Seedling vigor is maximum in distilled water treated seeds and is minimum in smoke water treated seeds (**Table1**).

Table 1 Comparison between Distilled and Smoke water Treated Seeds

TW	ARL	ASL	PG	SV
DW	1.764	0.441	89.0 %	196.245
SW	0.088	0.022	56.0 %	6.16

Where,
TW-Type of Water
ARL-Average Root Length
ASl-Average Shoot Length
PG-Percentage of Germination
SV- Seedling Vigor
DW – Distilled water treated seeds
SW –Smoke water treated seeds

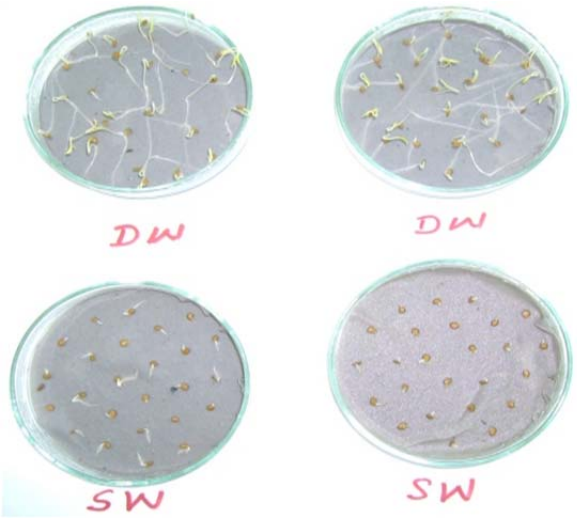

Fig.2 Difference in Seed Germination

Identification of Inhibitory Proteins

The gel obtained after running the SDS-PAGE with, Marker (Bovine Serum Albumin), smoke solutions treated seeds sample, normal tomato seeds sample and with distilled water treated seed samples in lanes 1, 2, 3 and 4 respectively. Thus, by observing at the bands obtained we can know that the bands 3, 5 in the second lane, the bands 6, 7 in the third lane and the band 8 in fourth lane are commonly present in all the three seed samples (**Fig.3**).

As the delay of germination observed in the aqueous smoke solutions treated seed samples, in second lane two new bands of probable molecular weights 63.87 Kda and 46.04 Kda were observed which were proposed to be the inhibitory proteins responsible for delay in seed germination (**Table 2**).

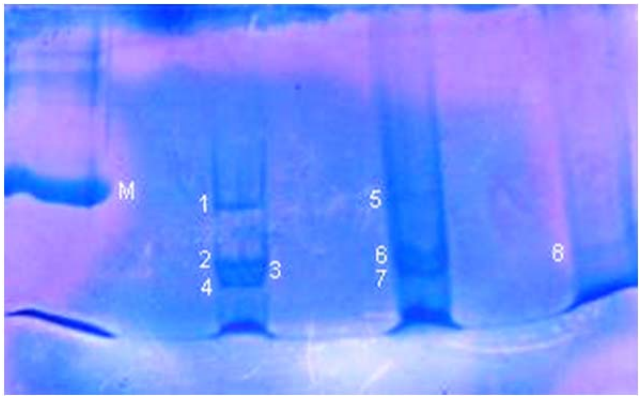

Fig.3 SDS-PAGE analysis for identifying Inhibitory Proteins

Table 2 Band pattern by SDS-PAGE analysis

Lane	Sample	Band	Rm Value	Probable Molecular Weight (KDa)
1	BSA	1	0.60	66.00
2	SWS	2	0.62	63.87
		3	0.80	49.50
		4	0.82	48.29
		5	0.86	46.04
3	NWS	6	0.80	49.50
		7	0.86	46.04
4	DWS	8	0.80	49.50

CONCLUSION

The effect of aqueous smoke solutions on the tomato seeds was observed physiologically by conducting the test of percentage of seed germination and seedling vigor tests by moist petri plate method. It is known that, every aspect shows an effect when a change occurs at its genetic level and gene expression. Here, as the aqueous smoke solutions treated tomato seeds have shown a delay in the seed germination compared to the distilled water treated seeds, which may be due to the change that has occurred in its gene expression. The expression of genes yields different kinds of proteins. So, some molecular biological tool like SDS-PAGE has been conducted, where the expression of genes can be observed at protein level by isolating the proteins produced. Thus, after running the SDS-PAGE, from the obtained results it can be concluded that the aqueous smoke solutions treated seeds have expressed two inhibitor proteins whose probable molecular weights are of 63.87 Kda and 46.04 Kda.

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