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MORPHOLOGICAL EVALUATION TRIGONELLAFOECUMGRAECUM UNDER THE INFLUENCE OF COLCHICINE WITH SPECIAL REFERENCE OF ITS VEGETATIVE CHARACTERISTICS

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ABSTRACT

Trigonellafoecumgraecum (TFG), commonly known as methi, comes under leguminous crops and self pollinating. India is the leading producer of TFG in the world. It is orally consumed as a leafy vegetable. It has been used as a medicinal plant since more than 4000 years in various parts of the world. Due to this reason, it is regarded as oldest medicinal plant in the history of mankind. An experiment was conducted to investigate the morphological changes through different colchicine treatments. The seedlings developed from treated seeds with colchicine at concentration range (0.2-0.6%) showed variations in plant growth (plant height, stem diameter, number of leaves, leaf length, leaf width, root length and number of lateral roots). The result of the present study demonstrates that at lower doses (0.2 and 0.4), significant changes in morphological characteristics were obtained. Among various concentrations 0.6% was found highly effective in all morphological characters. There was significant reduction in percentage germination in all concentrations as compared to the control.

Keywords: Trigonellafoecumgraecum, colchicine, morphological characteristics, seed germination.

Introduction

Fenugreek (*Trigonellafoenum-graecum, TFG*) is an annual legume crop belonging to the family of Fabaceae. It is native to an area extending from Iran to Northern India. The crop has now been introduced to parts of Africa, Mediterranean Europe, West and South Asia, North America, South America and parts of Australia (Acharya et al 2006 &Acharya et al 2008). India, is the leading producer of fenugreek in the world. It is best known for the presence of pungent aromatic compounds in their seeds that gives colour, flavour and aroma to food. It is orally consumed as leafy vegetable. It has been used as a medicinal plant in various parts of world. Due to this region, it is regarded as oldest medicinal plant in history of mankind (Bano et al 2016). Seeds of fenugreek spice have medicinal properties such as hypocholesterolemic, lactation aid, antibacterial, gastric stimulant for anorexia, antidiabetic agent, galactogogue, hepatoprotective effect and anticancer. The beneficial physiological effects of fenugreek including the antidiabetic and hypochlesterolemicactivity which are mainly attributable to the intrinsic dietary fibre constituent which have promising nutraceutical value (Srinivasan, 2006).

Polyploidy is widely acknowledged as a major mechanism of adaptation and speciation in plants (Osborn et al 2003). Chromosome doubling is a critical step in producing polyploids. Colchicine is the most frequently used chemical to produce autotetraploids in economically important crop species (Reinbergs&Shabeski 1958). The drug inhibits the formation of microtubules by binding to tubulin, the protein subunit of microtubules with the inactivation of spindle which is formed by microtubules, the polar migration of chromosome is inhibited producing "restitution" nuclei, thus resulting in a cell with a doubled chromosome number (Wan et al 1991). There are several methods for polyploidy induction by colchicine treatments in plants such as seed (Hanzelka&Kobza 2004, Quan et al 2004), flower bud (Wu et al 2007), apical meristem (Lavania&Srivastava 1991, Saharkhiz 2007) and root treatments (Taira et al 1991).

The colchicine concentrations usually applied ranged from 0.1 – 0.8% (Adaniya et al 2001). Keeping these facts in consideration, the present investigation was carried out to find out the response of *Trigonellafoenum-graecum* against various concentrations of colchicine.

Materials and Method

1. Plant material and growth conditions

   Seeds of *Trigonella*were purchased from local market of Jaipur, Rajasthan, India

2. Treatments

   Seeds of *Trigonella*were surface sterilized with 0.1% (W/V) $HgCl_2$ for 10 minutes. They were washed several times with distilled water to remove excess of $HgCl_2$. Seeds were soaked in distilled water for 24 hours. After this the pre-soaked seeds were subjected to varying concentration (0.2%, 0.4% and 0.6%) for 6 hours and control was maintained by pre soaking in distilled water.

   The soaked seeds were taken washed under running tap water. 30 seeds of each treatment were sown in the field following randomized block design (RBD) with three replcations along with control.

3. Growth measurements

   Growth measurements, (Table 1 & Table 2), for the plants exposed to colchicine and control, were taken after 15 days and 21 days of germination. The replicates taken for each treatment and control were used to calculate mean of each measurements.

Results & Discussion

Chemical mutagens have been reported to have inhibitory effects on seed germination leading to low percentage germination (Dhakhanamoorthy et al. 2010, Pande and Khetmalas, 2012). The percentage of seed germination was reduced on increasing doses of colchicine. Reduction in germination and survival percentages due to the effect of mutagens on various crop plants have earlier been documented (Mensah2005, Mensah&Akomeah 1997and Mensah et al 2005).

After 15 days of germination

In the control group plant height was 14.7 cms. It was observed that different concentrations of colchicine treatments (i.e. 0.2%,0.4% & 0.6%) employed in the present study succeeded in affecting the plant height. The effect of colchicine on different growth parameters are presented in table 1. The results revealed that increasing the concentrations of colchicine led to decrease in the plant height, leaf length, leaf width, root length and fresh weight. Control plants showed growth of nine lateral roots while treatment with colchicine led to absence of any lateral root development (Figure 1 A).

After 21 days of germination

The number of surviving plants on the 21st day followed similar trends as that of germination of earlier plants. Plants treated with 0.6% of colchicine had lowest germination percentage i.e. only 40%. These plants had better growth rate in comparison to the plants treated with other concentrations. These plants attained the height of about 11.96 cms. which is maximum among all concentrations.

A comparison of the control plants with the plants treated with colchicine is shown in Table 2. Plants treated with 0.6% colchicine produced more leaves with reduced leaf length and width and long roots (Figure 1 B).

Colchicine application reduced germination percentage significantly. Similar results were obtained by Lepengue et al (2012) and Sourour et al (2014). They showed a reduction in germination percentage of about 30.55% and 43.42% in Zea mays and barley respectively. Similar findings were obtained by Hassein et al (2001) and Bakry et al (2007) in Musa accuminata and Viciahardonensis respectively. It was found that percentage of seed germination decreased with increasing the doses of colchicine. In addition, among the surviving seedlings some were noticed to gradually die, especially those seedlings receiving high colchicine doses.

A linear trend between concentration of colchicine on germination and survival rate was observed. The mortality appeared to be due to poor seedling vigour resulting in inability of seedling to overcome the toxic effect of colchicine (Zlesak et al 2005). Addink (2002) stated that high concentration of colchicine could inhibit the development of living part resulting in mortality of organism. Generally, an average plant height is directly proportionate to the time period of growth. As a matter of fact, plant height decreased with increment in concentration of colchicine (Tiwari and Mishra 2012). Trigonella plants induced with colchicine treatment were visibly shorter and had broader stem. They had reduced number of leaves and shorter leaves. Treated plants are easily characterized by plant height, root length and number of lateral roots. Similar observations were reported by Nigel et al (2007), Grouh et al (2011) and Sourour et al (2014). Seed treatment with higher concentration of colchicine solution was noticed to cause the treated seeds to give low height plants. It works by disrupting the polymerization of microtubules which in turns disrupts spindle fibre development in mitosis. Cells arrested at metaphase may recover and enter the mitotic cycle

with twice as many chromosomes (Zlesak et al 2005). Jensen (1974) mentioned that in addition to the negative side effects of colchicine such as mitotic irregularities, growth retardation etc., other mutagenic effects including quantitative changes have been reported for various crops. Polyploid plants usually have thicker roots and shoots (Rose et al 2000).

After 21 days of germination, 0.6% concentration of colchicine treatment supports the plant height, root length and number of leaves. Essel et al (2015) reported that colchicine Induced marked vegetative growth, leading to the formation of large plants and more number of leaves, branches and seeds per plant. These characters were highest as compared to other concentrations. It could be further used and be helpful in breeding programs.

In conclusion, colchicine application reduced germination percentage. Increasing ploidy often results in increasing cell size that in turn results in thicker stems and broader leaves. Shoots are often thicker and can have shortened internodes. Morphological variations directly correlated with concentration of colchicine.

Table1: Morphological parameters after 15 days of growth.

S.No	Growth Parameters	Control	0.2%	0.4%	0.6%
1	Plant length (cms)	11.7±0.05	6.95±0.50	4.43±1.01	5.43±0.04
2	Stem diameter (cms)	0.1±0.07	0.16±0.02	0.26±0.05	0.25±0.04
3	No. of leaves	3±1.02	2±0.05	2±0.05	2±0.07
4	Leaf length (cms)	2±1.00	1.06±0.07	0.93±0.05	0.90±0.57
5	Leaf width (cms)	0.7±0.69	0.48±0.54	0.45±0.41	0.42±0.30
6	Root length (cms)	5.3±1.25	2.93±0.91	2.48±1.01	0.93±0.94
7	No. of lateral roots	9±0.05	0	0	0
8	Fresh weight	0.21±0	0.12±0.04	0.12±0	0.11±0.04
9	Germination%	100	94	80	73

Table 2: Morphological parameters after 21 days of growth.

S.No	Growth Parameters	Control	0.2%	0.4%	0.6%
1	Plant length (cms)	16.8±0	6.71±0.05	4.8±0	11.96±0
2	Stem diameter (cms)	0.2±0.01	0.23±0.02	0.25±0	0.35±0
3	No. of leaves	6±0	3±0	4±0	5.2±0.02
4	Leaf length (cms)	2.1±0.05	1.17±1.04	0.96±0.96	0.73±0.58
5	Leaf width (cms)	1.5±0	0.70±0	0.65±0	0.47±0.06
6	Root length (cms)	5.3±1.25	1.83±1.06	1.58±0	3.13±.63
7	No. of lateral roots	6±0	1±0	1±0	0
8	Fresh weight	0.28±1.02	0.14±1.25	0.11±0	0.30±0.90
9	Germination%	100	81	78	40

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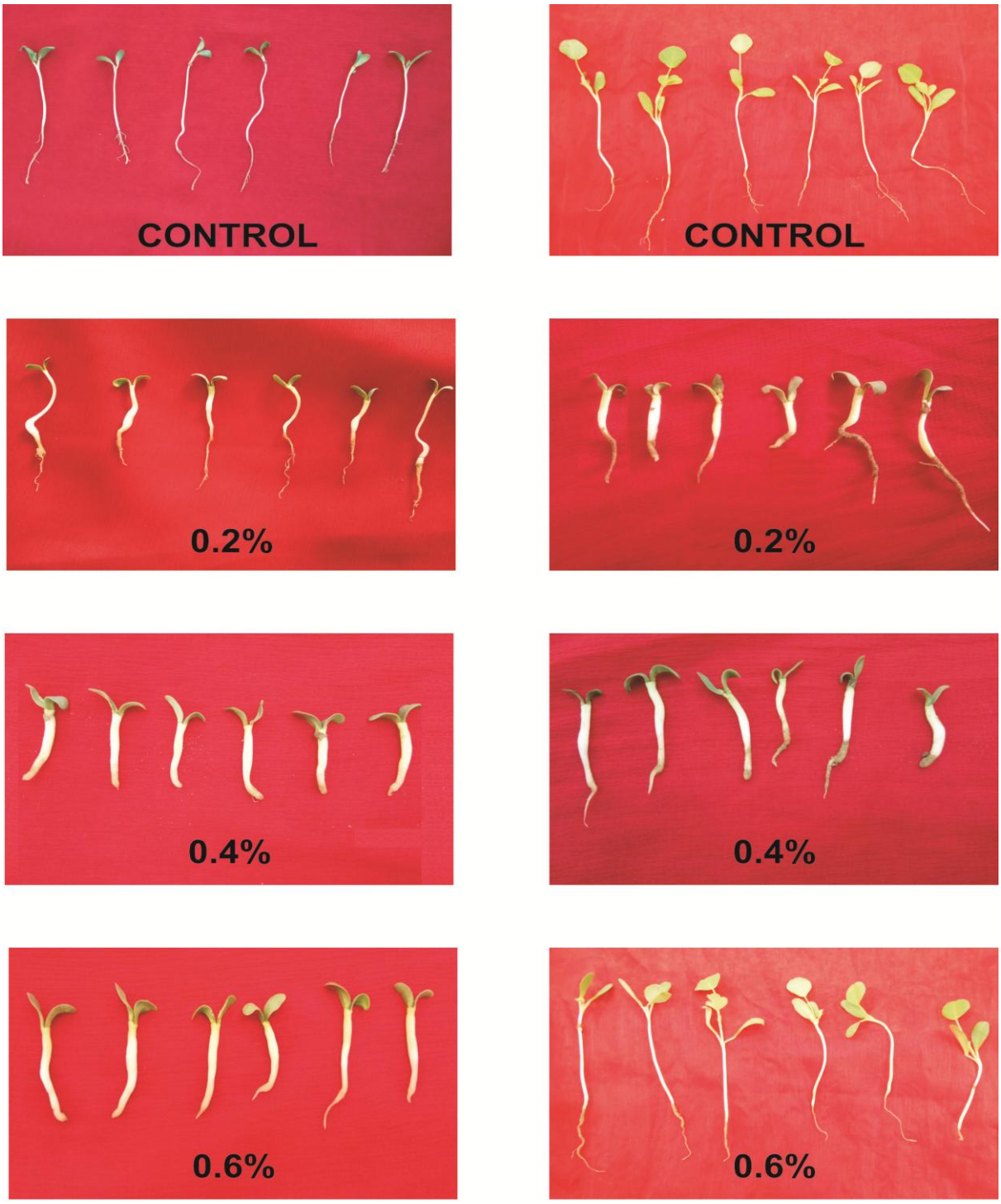

Figure 1-A) Seedlings after 15 days of germination
B) Seedlings after 21 days of germination