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ORIGINAL ARTICLE

Effect of Ethanol on Germination and Enzyme Activities in Finger millet (*Eleusine coracana* Gaertn.) Seeds

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Influence of ethanol the end product of alcoholic fermentation on the growth of finger millet (var. GPU-28, CO-9) seedlings of two finger millet was studied as a means of evaluating growth responses under anoxia. The germination was delayed by ethanol treatment in case of both the cultivars. Ethanol treatment affected the growth of both radicle and coleoptile of seedlings. In this respect the radicle growth is more sensitive to ethanol than the coleoptile in both varieties of finger millet. The activities of enzymes nitrate reductase, ATPase, acid phosphatase, amylase were reduced by alcohol treatment in germinating seeds of both the cultivars. However, lower concentration of alcohol (1%) caused stimulation of peroxidase in var. CO-9. In case of var. GPU-28 showed stimulation of enzyme alkaline phosphatase in both concentration of alcohol.

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It is well known that there is accumulation of ethanol in the plant tissue under waterlogged conditions and it is quite possible that some of this accumulated ethanol is released in the surrounding medium and can exert an allelopathic influence. Ethyl alcohol is the major product of anaerobic fermentation in most of the seeds germinating under anaerobic conditions and this process has been largely exploited for production of alcoholic beverages. However the effect of such an anaerobically produced ethanol on the seedling growth or seed metabolism has been investigated by very few workers. Among all the cereals, rice seeds have greater ability to germinate and grow under extremely low oxygen conditions even under vacuum (Perata *et al.,* 1997, Setter *et al.,* 1997 and Vartapetian and Jackson 1997). Such studies are

mainly carried out on rice which is displays maximum tolerance to waterlogging stress (Hypoxia and Anoxia). In the present investigation an attempt has been made to study the effect of exogenously applied ethanol on the growth of the seedling and behavior of some enzymes in the finger millet seedlings. For this purpose, a red cultivar (GPU-28) and white grain cultivar (CO-9) has been taken. It is reported by Kono *et al.,* (1988) that finger millet (*Eleusine coracana* Gaertn.) possesses good waterlogging potential.

MATERIALS AND METHODS

Seeds of finger millet variety GPU-28 (red grains) were collected from Agriculture Research Station, Shendapark, Kolhapur (MS) and variety CO-9 (white grains) were collected from department of Millets, Coimbatore (TN). Two concentrations of ethanol 1% and 6% were chosen for study after preliminary screening .Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 min. Afterwards, seeds were rinsed with distilled water for 4-5 times. For germination study 10 seeds in each Petri plate were placed on the filter paper moistened with the respective alcohol solution .Seeds placed on filter paper moistened with distilled water served as control. Seeds were allowed to germinate in room temperature in dark.

For metabolic studies 500 mg seeds were allowed to germinate in large Petri dishes on respective solutions at room temperature under laboratory conditions. For study of enzyme α-amylase and acid phosphatase the plant material was homogenized in acetate buffer pH 5 while for extraction of enzyme peroxidase Phosphate buffer pH- 6.8 was employed. The extraction medium described by Weimberg (1970) was used for extraction of enzyme ATPase and alkaline phosphatase. The activity of enzyme amylase was studied according to Katasumi and Fukuhara (1969). The activity of acid phosphatase was assayed according to method of McLachlan (1980). To study peroxidase activity method of Horiguchi (1988) was followed. The activity of enzyme ATPase was determined according to the method described by Todd and Yoo (1969). The activity of enzyme alkaline phosphatase was studied following the method of Weimberg (1970). Soluble proteins in the enzyme extracts were estimated by the method described by Lowry *et al.,* (1951). Activity of nitrate reductase was determined following the *in vivo* method described by Jaworski (1971).

RESULTS AND DISCUSSION

The influence of ethanol treatment on germination percentage of the two finger millet cultivars is reduced in Fig. 1a and Fig. 1b. The germination is retarded by ethanol treatment and there is great drop in germination percentage at 24 hr stage. At latter stages there is a recovery in seed germination in both cultivars. An inhibitory effect of ethanol on seed germination is recorded by some workers. In the waterlogging tolerant *Echinocloa* reported marked inhibition of seed germination due to 1% ethanol treatment. Present investigation indicates that 1% ethanol is not causing any reduction in germination percentage in finger millet seedlings. However the seedling growth in terms both radicle and coleoptile growth were affected by 1% ethanol especially in the red seeded cultivar (Table 1a). Vigour index of red

seeded variety was more declined than white seeded (Table 1b). Our finding recalls the work of Kato-Noguchi and Kuginiya (2001) who reported that the elongation of roots and coleoptiles of the rice seedlings was inhibited by ethanol, at concentrations about 100mM and 200mM. The inhibitory effect of ethanol on finger millet seedling growth is highly significant at 6% ethanol concentration. Hence the enzymatic studies have been performed by giving treatments of 1% and 6% ethanol to the finger millet seeds. Since the first 24h represent a crucial period of seed germination the study of enzymes has been performed at this germination stage.

Influence of ethanol on α-amylase activity in the germinating seeds of finger millet is depicted in Fig. 2a. Ethanol treatment has caused marked decrease of enzyme activity in GPU-28 and slightly decrease of the same in cultivar CO-9. Lower concentration of ethanol is highly effective in this respect. Among various enzymes operating during seed germination in cereal grains amylase are the most important since these are involved in breakdown of the major carbohydrate reserve starch. The regulation of α-amylase synthesis by gibberellin and the inhibition of GA action by Abscisic acid are very well documented in cereal grains. The activity of amylase in germinating seeds is also affected by various environmental constrains such as salinity and water stress (Zayed and Zeid 1997, Gupta *et al.,* 1993). It is reported that under improved anoxic conditions the activity of this enzyme is lower (Yi *et al.,* 2002). Our findings indicate that such effect is probably brought about by the ethyl alcohol produced in the cells. The inhibition of α-amylase by alcohol treatment would certainly limit the availability of substrate for respiration in germinating seeds.

The acid phosphatase and alkaline phosphatase display opposite response to ethyl alcohol treatment during early stage of finger millet seed germination (Fig. 2b and Fig. 3a). The activity of enzyme acid phosphatase is reduced due to alcohol treatment such reduction is more marked in a red seeded variety GPU-28. The activity of alkaline phosphatase is increased in the seeds of variety of GPU-28 and seed of cultivar of CO-9 treated with 1% ethyl alcohol. The hydrolytic enzyme acid phosphatase is reported to utilize several phosphate substrate in the plants (Vincent *et al.,* 1992) and the activity of this enzyme shows an increase during the case of seed germination (Tamura *et al.,* 1982). The inhibition of this enzyme can certainly cause a disturbance in phosphorus metabolism to some extend this can be compensated by relatively stable alkaline phosphatase activity.

The activity of enzyme ATPase is considerably affected by ethanol treatment, in case of cultivar GPU-28 (Fig. 3b). On the other hand, in case of cultivar CO-9 such effect is noticeable in the seeds treated with 6% ethyl alcohol. ATP is a biological energy currency and the ATP hydrolysis provides energy for various metabolic activities as well as growth processes in the cell. Hence, the inhibition of ATPase enzyme by ethanol can cause for reaching effect on the growth and overall metabolism of the germinating seeds.

Activity of enzyme nitrate reductase is greatly affected by ethyl alcohol in finger millet cultivar GPU-

28 and in cultivar CO-9 (Fig. 4a). Nitrate reductase is one of the major enzymes of nitrogen metabolism which catalyses the primary step of nitrogen assimilation in higher plants. The inhibition of this enzyme would not only affect nitrate reduction but disturb the overall the nitrogen metabolism in the germinating seeds.

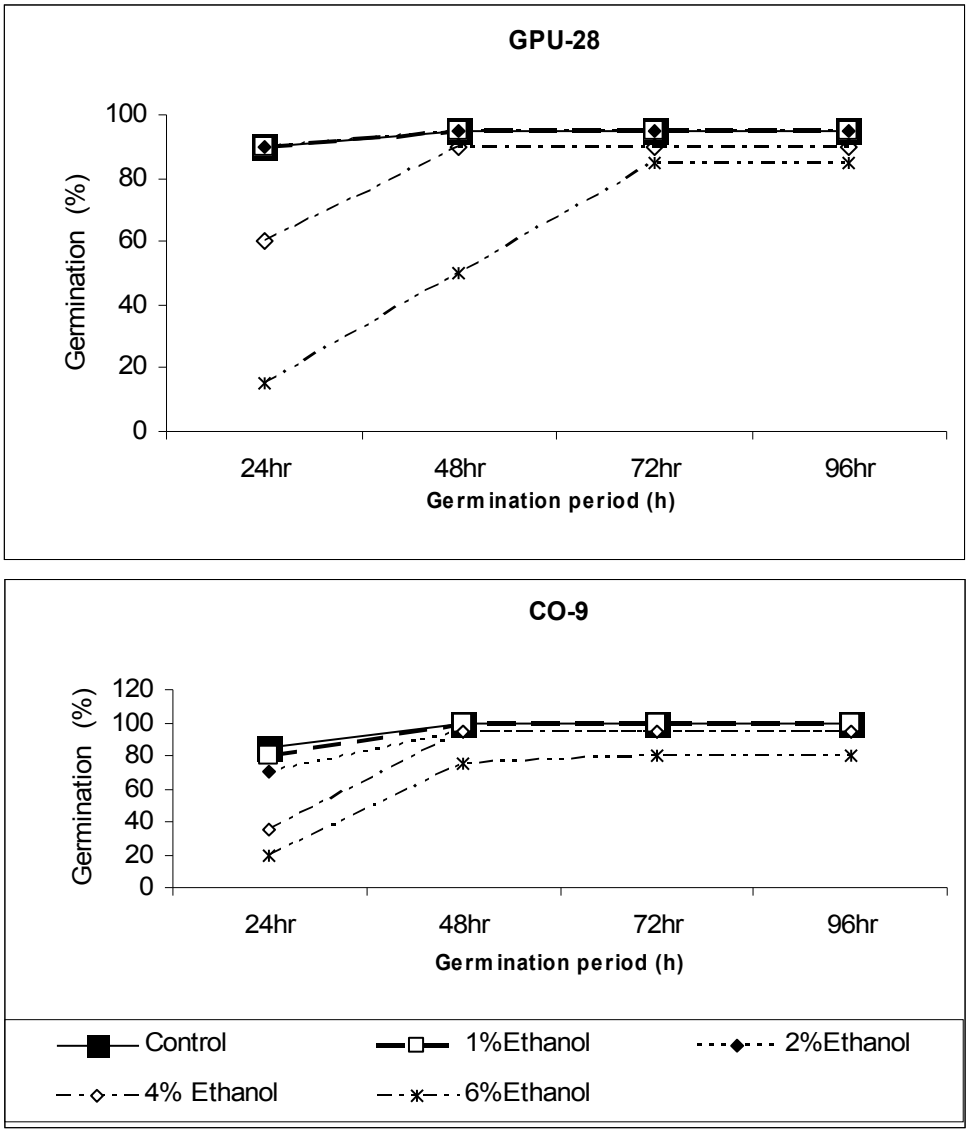

Figure 1. Influence of Ethanol treatment on seed germination in fingermillet cultivars GPU-28 (a) and CO-9 (b).

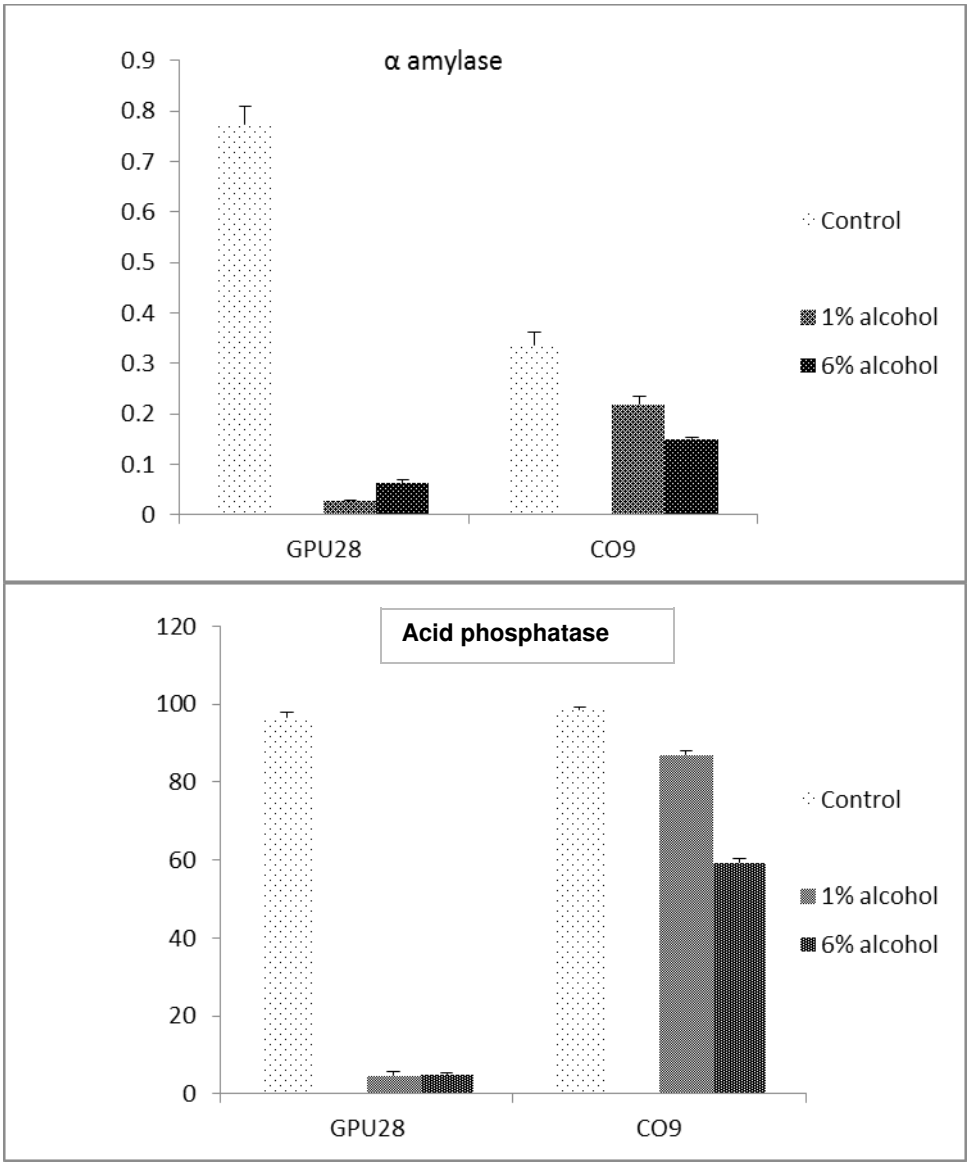

Figure 2. Influence of Ethanol treatment on activity of enzyme amylase (a) and acid phosphatase (b) during 24 germination in two fingermillet cultivars GPU-28 and CO-9

Table 1a. Influence of Ethanol treatment on seedling growth in finger millet cultivars GPU-28 and CO-9

	Control radicle	Control coleoptile	1% ethanol radicle	1% ethanol coleoptile	2% ethanol radicle	2% ethanol coleoptile	4% ethanol radicle	4% ethanol coleoptile	6% ethanol radicle	6% ethanol coleoptile
GPU28 mean	2.57	1.67	1.13	0.75	0.48	0.29	0.1	0.13	0.1	0.1
S.D	0.312	0.309	0.099	0.212	0.122	0.0875	2.48E-09	0.0483	2.48E-09	2.48E-09
CO-9 Mean	1.71	1.15	1.64	0.94	0.95	0.43	0.13	0.21	0.1	0.12
S.D.	0.15	0.32	0.128	0.22	0.32	0.082	0.048	0.087	2.48E-09	0.0421

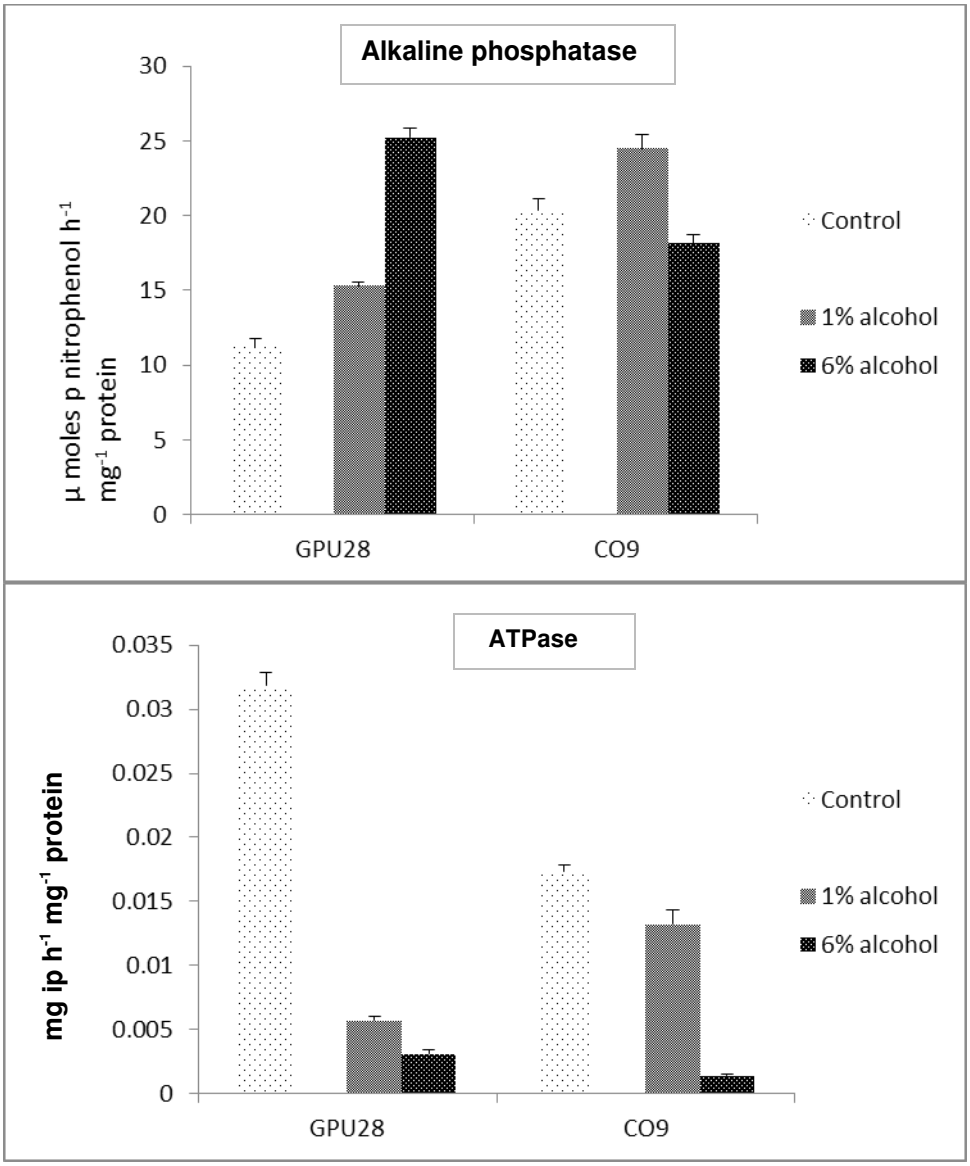

Figure 3. Influence of Ethanol treatment on activity of enzyme alkaline phosphatase (a) and ATPase (b) during 24 germination in two fingermillet cultivars GPU-28 and CO-9

Table 1b. Influence of Ethanol treatment on vigour index in finger millet cultivars GPU-28 and CO-9

	Control	1% ethanol	2% ethanol	4% ethanol	6% ethanol
GPU-28	402.8	178.6	73.15	20.7	17
CO-9	279	265	131.1	32.3	17.6

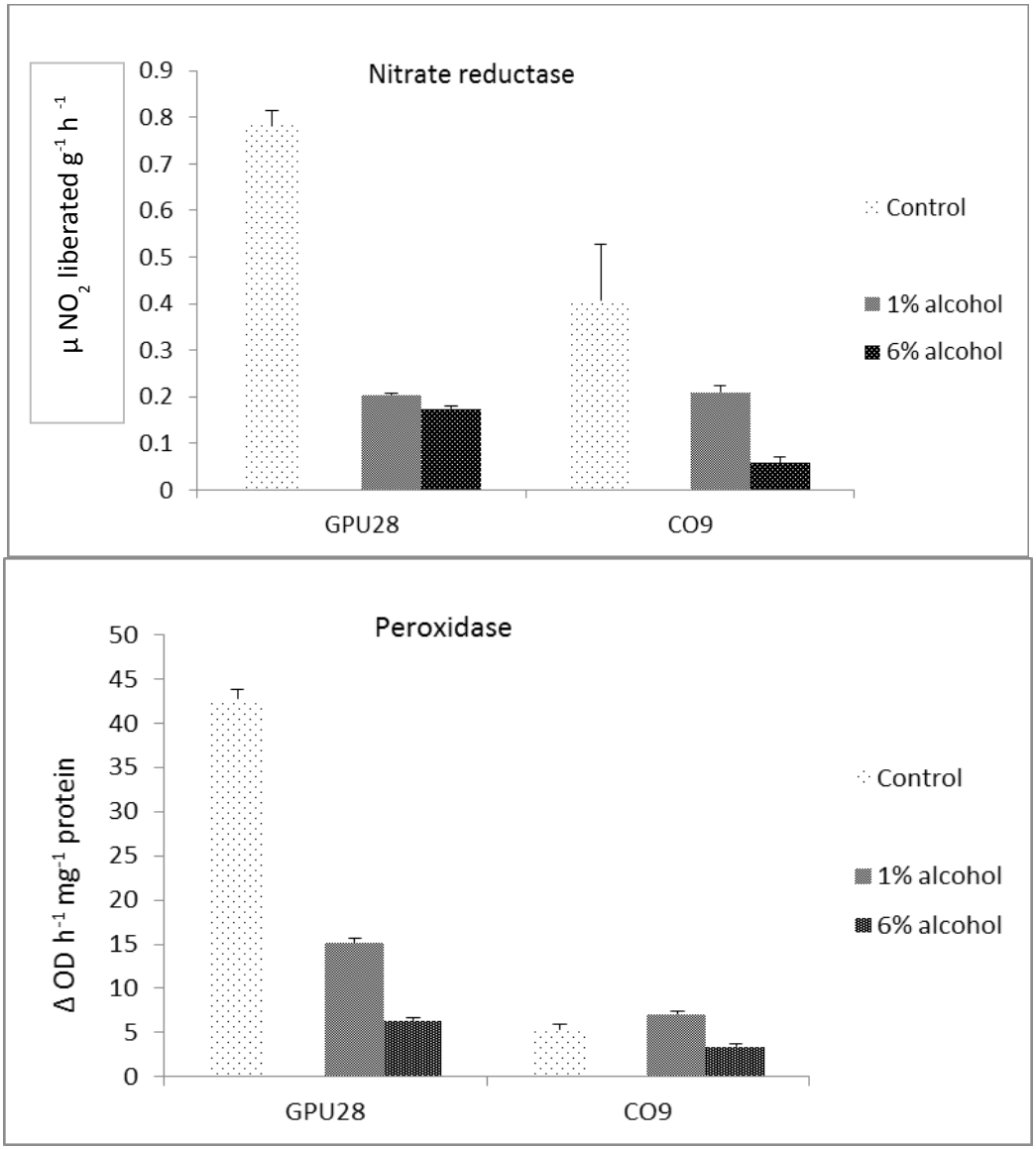

Figure 4. Influence of Ethanol treatment on activity of enzyme nitrate reductase (a) and peroxidase (b) during 24 germination in two fingermillet cultivars GPU-28 and CO-9

Activity of enzyme peroxidase is also considerably reduced due to ethanol treatment in cultivar GPU-28. In case of cultivar CO-9 the inhibition of the enzyme activity is noticeable at 6% ethyl alcohol treatment (Fig. 4b). Peroxidase is reported to be involved in the scavenging of free radicals generated due to various stress factors (Kim *et al.,* 1999). Further the enzyme is also participates in the secondary metabolism leading to lignification and cell wall biogenesis (Ingham *et al.,* 1998). The inhibition of peroxidase by ethanol would affect both these aspects in the germinating finger millet seeds.

It is evident from the foregoing account that ethanol treatment affects activities of some important enzyme systems during initial phase of seed germination and such effect is more prominent in the red seeded variety GPU-28 than white seeded cultivar CO-9. Such alteration in the enzyme activities would undoubtly affect the overall growth of the seedlings.

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