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EFFECT OF TEMPERATURES AND VARIOUS GROWTH REGULATORS ON SEED GERMINATION OF TWO VARIANTS OF *CHENOPODIUM ALBUM* L.

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Effects of temperatures (10, 20, 30, 40 and 50°C) and various growth regulators i.e., IAA, GA_3, Cytokinin, ABA and ethylene on percentage seed germination of two variants of *Chenopodium album* were studied. For this purpose, *C. album* seeds were germinated under different temperature conditions and after treating with various hormone concentrations for 15 days. It was determined that both the variants have specific temperature requirements for optimum germination while the effect of growth regulators for optimum germination varies considerably in their kinds and strengths.

Keywords: *Chenopodium album;* Germination; Growth regulators; Seasonal ecotypes; Variants.

Introduction

Populations even within an environment showing heritable morphological differences can be shown and separated by ecological features of the differences in their micro environment. Trivedi[1] worked on *Utricularia stellaria* and found a photofuge growing adhering to the under surface of peltate leaves of *Nymphea* where as a photocole ecotype growing in water directly under the sun. These are logically called as genuine photo-ecotypes.

Although much information is not available on climatic races from different geographical regions, beginning with Turesson[2,3], Clausean *et al.*[4,5] and McMillan[6] showed the role of temperature and photoperiod in the geographical distributions of climatic races. Almost no work exists, regarding the populations from same area which are adapted to seasonal differences in respect of temperature conditions. However, Kaul[7] has shown the existence of seasonal ecotypes of *Xanthium strumarium* L. differing in respect of morphological characters such as size of leaf and height of plant in response to photoperiod.

In the present study it has been observed that two populations of *Chenopodium album* L. differing in certain minor but constant morphological characters though with similar floral characters but separated by seasons only. One is growing during the hot rainy season (June to September) and the other during winter (November to February). These show some minor inheritable differences in their morphology, as well as ecology, especially rate of growth and overall size of plants but in floral structures they are identical. Also there are no edaphic preferences of the two populations because both grow on the same sandy soil of Jaipur. In view of the above description the present work is done.

In the present work seed germination studies were carried out for both summer and winter variants of *C.album* for comparison and in order to find out their taxonomical relationship based on ecology. The seeds of *C.album* are generally dormant at maturity, although the percentage of dormant seeds varies considerably among different seed lots[8]. Dormancy of the species can be broken by various chemical and physical treatments[9-11]. Germination is generally defined as those events that commence with the imbibition and terminate when the radical (embryonic root; or in some seeds, the cotyledon / hypocotyls) elongates and emerges through the seed coat[12,13]. A seed remains viable but unable to germinate or grow for several reasons.

Numerous factors influence the processes of seed dormancy and germination. Seeds consist of a small embryonic axis along with some storage tissue, enclosed by a series of coverings called the seed coat. The seed coat serves the protective function as much as bud scales do for the bud. Its presence often suppresses germination by restricting the uptake of water and exchange of oxygen. Seed coat mechanically limits the expansion of embryo and in some cases it contains inhibitors that prevent the growth of embryo. Seed dormancy refers to the situation

wherein the seed fails to germinate or grow because of some physiological or environmental limitations. The limitations commonly include the inability of water or oxygen to penetrate the seed coat. Measurements of the oxygen and permeability of seed coats have been made and there is general agreement that permeability is very low in the dormant seeds[14].

The seeds of *C. album* L. are lens shaped with curved embryo, endosperm is absent though perisperm is present[15]. The seeds of different populations of *C. album* are dormant for varying periods. A germination inhibitor is reported by Watanabe[16] in the seeds of *C. album* which retards the germination.

According to Williams and Harper[17] addition of nitrate along with ethylene treatments increase the germination percentage of seed. Saini[11] also proved that manipulation of the nitrate content of seeds modulates the dormancy breaking effect of ethylene on *C. album.* Light plays very important role in the initiation of radical growth that is to say that the seeds of *C. album* are photoblastic.

Material and Methods

In *C. album* L. fruiting in the winter population and summer population occurs in the month of the February-March and September- October respectively. Seeds for both the variants are collected seasonally for morphological and analytical work. Seeds were stored in glass stoppered bottles. After preliminary selection for uniformity crieteria (size and colour of seeds), the seeds were surface sterilized with 0.1% $HgCl_2$ for two minutes, then washed with distilled water three times[18].

The germination tests were performed by placing the *C. album* seeds in Petri plates on moist filter paper. Usually 15 seeds were used with three replicates. Germination of seeds were recorded for a period of 15 days[19]. Germination was considered as the penetration of the radical through the seed coat. The studies were carried out all round the year besides their growing seasons *i.e.,* summer and winter. All the results were tabulated and analyzed by employing F- test at 1% and 5 % level of significance[20,21].

For hormonal treatments, seeds were soaked in the hormones, namely, Indole Acetic Acid, Gibberellic Acid, Cytokinin, Ethylene and Abscisic Acid at various concentrations for varying duration. Seeds were soaked in the distilled water used as the control.

Seeds were watered for keeping them moist at regular intervals. The Petri dishes were kept under laboratory conditions of temperature and light. The data were represented in ultimate form of percentage germination.

Result and Discussion

All data regarding the effects of temperatures and growth regulators on seed germination percentage of summer and winter variant of *C. album* are recorded in the Tables 1-4 and Figs. 1-3, respectively.

Effect of Temperature- Temperature is the most important external factors in controlling seed dormancy and germination[22].

In the summer variant the seed germination percentage declined when seeds were kept at 10°C for 24 hrs (Table1) and onwards while in the winter variant enhanced germination percentage was recorded when seeds were kept at 10°C for 48 and 72 hours of treatment in comparison to control (Table 2). 10°C is found to be the optimum temperature for the winter variant and 30°C for summer variant for seed germination.

Germination percentage decreased up to certain extent in summer variant under all treatments. However, germination percentage improved significantly in the seeds of winter variant *vis-a-vis* control (13.3%) when treated for 48 hrs (24.4%) at 20°C. At 30°C the results were contrary to 10°C and 20°C treatments, there was increase in germination of summer variant *vis-a-vis* control 17.7 to 39.9% while a significant loss in germination was observed in the winter variant *vis-a-vis* the control 17.7 to 11.0% when treated for 48 and 72 hrs. The seed germination increased in summer variant up to 28.8% when compared with control *i.e* 8.8% for 36 and 48 hrs at 40°C. There was no significant change in the winter variant up to 48 hrs. However, when the seeds of winter variant were treated for 72 hrs there was no germination at all.

There was no significant effect of 50°C temperature after 24 hrs treatment on summer variant, the seed germination was 19.9% as compared to the control *i.e.,* 17.7% but subsequently there was a decline in germination percentage with increase of period of treatments. In case of winter variant there was no germination beyond 24 hrs of treatment. The germination was only 6.6% after 24 hrs of treatment in comparison to control where it was 22.1%.

When the data were statistically analysed the results were very highly significant at 10°C between variants. At 20°C significant results were found among replicates. Between the variants the results were found to be highly significant at 30°C. Control *vs* treatment and between variants the highly significant results were obtained at 40°C. At 50°C very highly significant results among control vs treatment and among periods significant results were obtained. Except these the results were not significant (CRD ANOVA Table 1 and 2).

Table 1. Effect of temperature on seed germination (%) of the summer variant of *Chenopodium album* (values are means of three replicates each).

Temperatures °C	Control	Time (Hours)			
		24	36	48	72
10	17.7	8.8	6.6	2.2	2.2
20	24.4	17.7	11.0	8.8	13.3
30	17.7	22.1	17.7	39.9	26.6
40	8.8	24.4	28.8	28.8	22.2
50	17.7	19.9	4.4	2.2	00

Table 2. Effect of temperature on seed germination (%) of the winter variant of *Chenopodium album* (Values are means of three replicates each).

Temperatures °C	Control	Time (Hours)			
		24	36	48	72
10	13.3	17.7	19.9	26.6	26.6
20	13.3	11.0	19.9	24.4	22.1
30	17.7	24.4	15.5	11.0	8.8
40	13.3	19.9	13.3	13.3	00
50	22.1	6.6	00	00	00

CRD ANOVA between variants and controls

F ratio	Control *Vs* treatment	Between variants	Among hours	Among replicates
10°C	0.932^{NS}	14.909***	0.035^{NS}	3.20^{NS}
20°C	1.09^{NS}	3.00^{NS}	0.201^{NS}	3.84*
30°C	2.925^{NS}	10.88**	1.47^{NS}	1.21^{NS}
40°C	14.29***	5.84*	1.59^{NS}	0.49^{NS}
50°C	105.23***	0.04^{NS}	4.0*	2.61^{NS}

NS = Not significant; * = Significant; ** = Highly significant; *** Very highly significant; CRD = Computerised random design

Effect of Various Growth Regulators (Plant Hormones)- Growth regulators play vital roles in the growth and development of plants. Some of the growth regulators play prominent role in seed germination while others do not. However, the effect is always interrelated with one another. Here the results are tabulated in Tables 3 and 4.

Indole Acetic acid (IAA)- Seed germination increased in the summer variant, *vis-a-vis* the control from 17.7% to 39.9%, 35.5% and 46.6% when treated with concentrations of IAA at 100, 500 and 1000 ppm. However, the effect of IAA at 10 and 50 ppm was not significant (Table 3). In case of the winter variant, seed germination percentage

Table 3. Effect of different growth regulators (plant hormones) on the seed germination (%) of the summer variant of *Chenopodium album* (values are means of three replicates each).

Growth Regulator	Control	Concentrations (ppm)				
		10	50	100	500	1000
Indole Acetic acid	17.7	19.9	28.2	39.9	35.5	46.6
Gibberellic Acid	19.9	24.2	33.3	44.4	55.5	53.3
Cytokinin	11.0	19.9	17.7	11.0	8.8	17.7
Ethylene	15.5	22.1	24.4	33.3	26.6	37.7
Abscissic Acid	8.8	17.7	22.1	22.2	22.1	24.4

Table 4. Effect of different growth regulators (plant hormones) on the seed germination (%) of the winter variant of *Chenopodium album* (values are means of three replicates each).

Growth Regulator	Control	Concentration(ppm)				
		10	50	100	500	1000
Indole Acetic acid	22.2	15.5	22.1	31.0	33.3	33.3
Gibberellic Acid	11.0	19.9	24.4	33.3	42.2	35.5
Cytokinin	19.9	22.1	28.8	31.0	19.9	24.4
Ethylene	19.9	28.8	33.3	35.5	35.5	28.8
Abscissic Acid	15.5	15.5	13.3	17.7	8.8	15.5

CRD ANOVA between variants and controls

F ratio	Control *Vs* treatment	Between variants	Among conc.	Among replicates
Indole Acetic acid	82.47***	0.87^{NS}	11.41***	2.35^{NS}
Gibberellic Acid	138.99***	31.19***	7.55***	4.67*
Cytokinin	8.44**	35.52***	0.88^{NS}	2.09^{NS}
Ethylene	49.66***	4.78*	0.76^{NS}	0.55^{NS}
Abscisic Acid	10.79**	0.02^{NS}	0.30^{NS}	1.42^{NS}

NS = Not significant; * = Significant; ** = Highly significant ; *** Very highly significant; CRD = Computerised random design

also increased with IAA treatment but it was comparatively less than in the summer variant i.e., from control 22.2% to 33.3% at both 500 and 1000 ppm concentration.

Gibberellic Acid (GA_3)- Gibberellic acid was found to be the most effective in increasing the germination percentage. In the summer variant germination increased from 19.9% to 55.5% and 55.3% when treated with GA_3 at 500 and 1000ppm concentrations, respectively. Germination in the winter variant also increased as compared to control 11.0% to 42.2% and 35.5% under treatment with 500 and 1000 ppm of concentrations, respectively. It was observed that lower concentrations

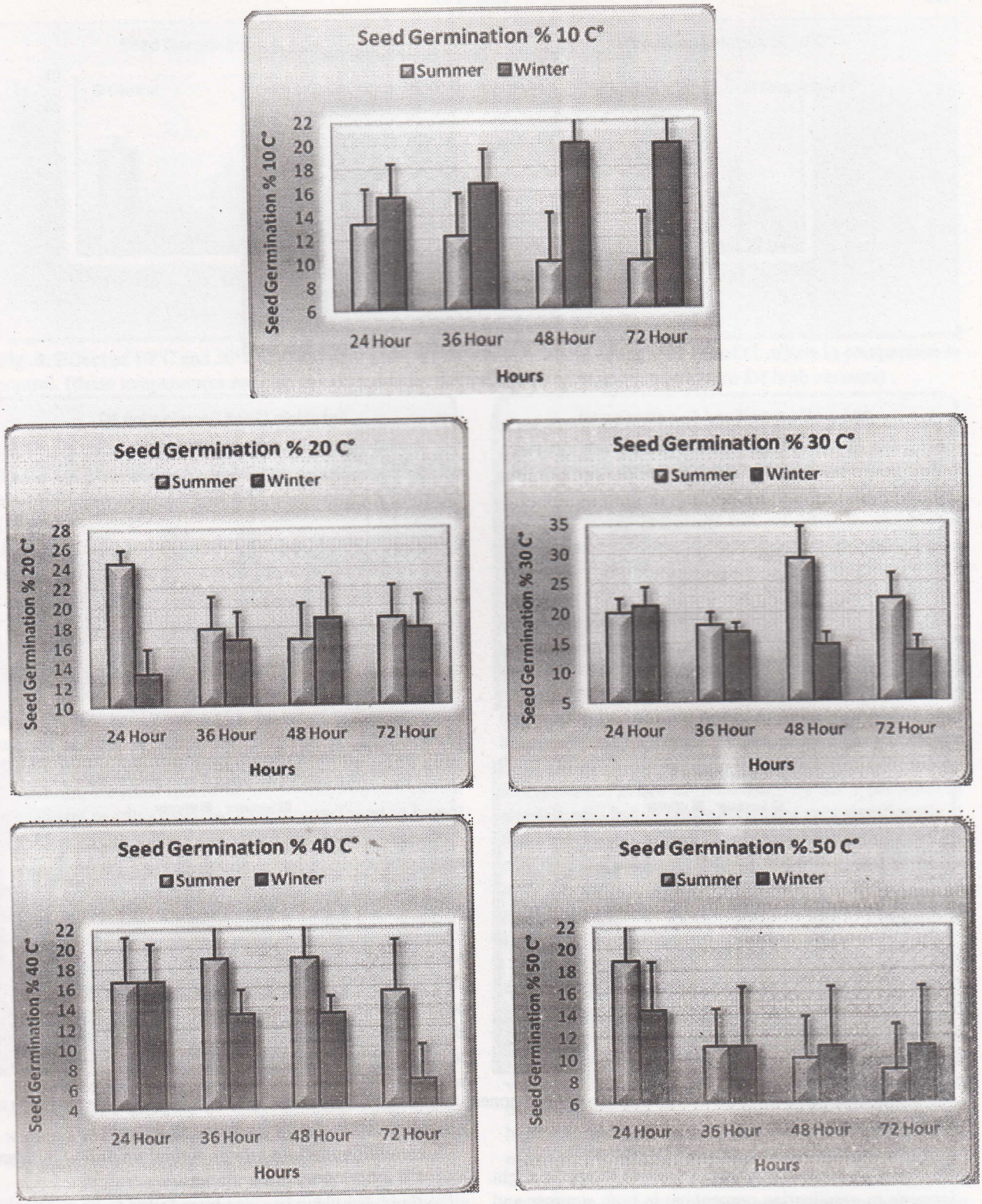

Fig.1. Effect of different temperatures on seed germination (%) of summer and winter variants of *C. album.*

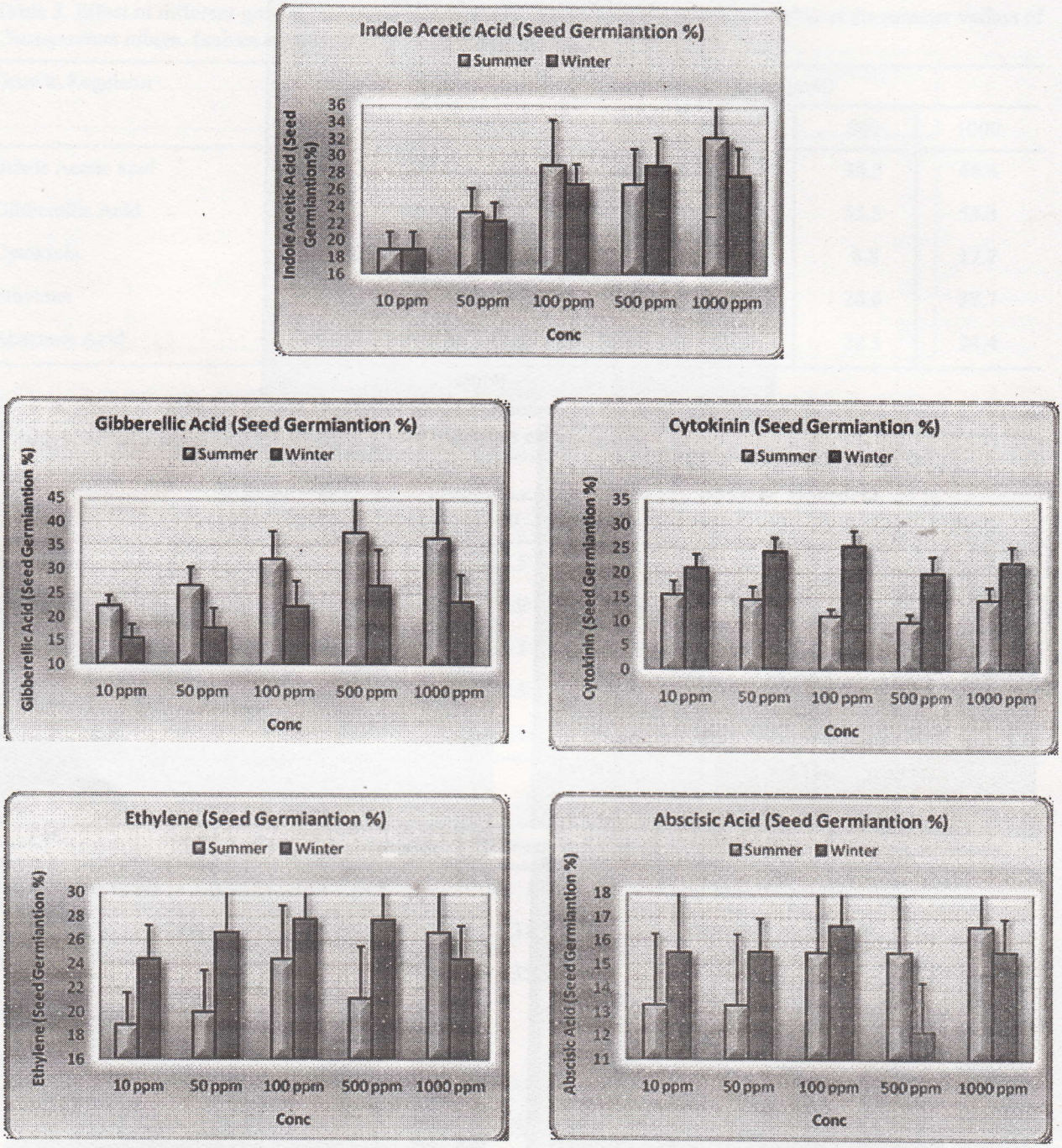

Fig.2. Effect of different growth regulators (plant hormones) on seed germination (%) of summer and winter variants of *C. album.*

were not much effective to promote germination.

Kinetin (Cytokinin)- Cytokinin treatment showed a slight increase in germination percentage in both summer and winter variant. In case of summer variant germination percentage was increased up to 19.9% at 50 ppm of concentration while in winter variant up to 31.0% when treated at 100 ppm concentration.

Ethylene- Ethylene has also been found to be promotive for seed germination but lesser than IAA and Gibberellic acid. Its application increased the germination up to 37.7%

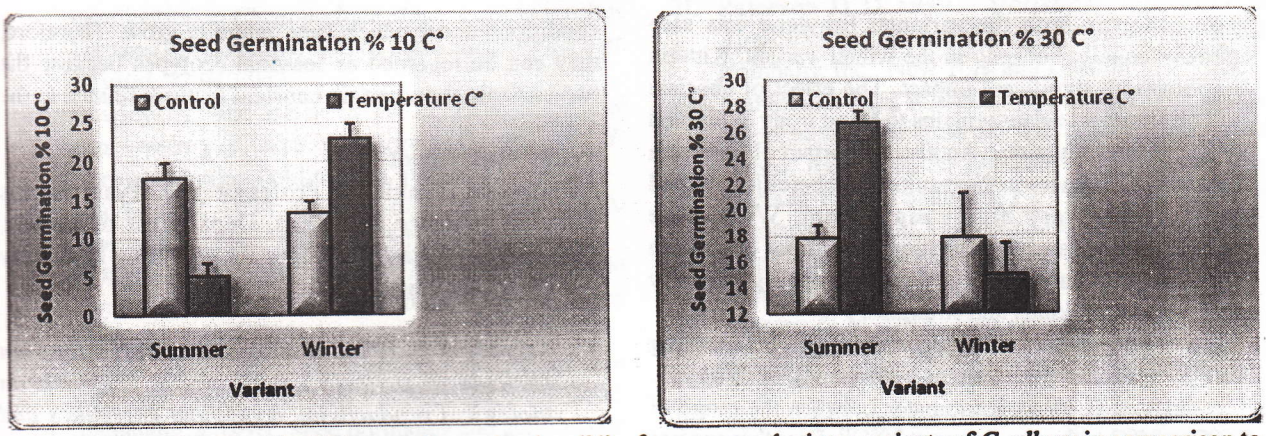

Fig .3. Effect of 10°C and 30°C on seed germination (%) of summer and winter variants of *C. album* in comparison to control. (these temperatures are also concluded to be the respective optimum temperatures for both variants) .

vis-a-vis the control 15.5% in the summer variant under 1000 ppm concentration. In case of the winter variant, the maximum germination (35.5%) was recorded under 100 and 500 ppm concentrations in comparison to control (19.9%).

Abscisic Acid- Percentage of seed germination increased in the summer variant up to 24.4% at 1000 ppm of concentration of Abscisic acid (Table 3). However, other concentrations of Abscisic acid were not higher in comparison to the control. In the winter variant the effects were negligible

When the statistical analysis was performed only the giberellic acid was found to be very highly significant among control *vs* treatment, between variants, among concentrations as well as replicates. Indole acetic acid, Cytokinin, ethylene and Abscisic aci showed one or two significant results otherwise results were found to be not significant (CRD ANOVA Table 3 and 4).

Many observations have been made on the effects of exogenous hormones in imposing or breaking dormancy[23]. The role of different plant hormones is inevitable for plant growth, however not all the hormones play significant role in the seed germination. A variety of chemicals are known to break the seed dormancy[12] but ethylene and nitrate are particularly found suitable for field applications. However, the effectiveness of these chemicals has been found to be highly variable and to be modified by environment. In the present study seeds of both the variants show breaking of seed dormancy when treated with Indole acetic acid, ethylene, gibberellic acid, kinetin and abscisic acid at various concentrations.

From the results of present study gibberellic acid was found to be the most promotive, followed by Indole acetic acid. Gibberellins are responsible for mobilization of reserve food material in the seed during germination. It enhances the activity of amylase which is crucial for germination. Indole acetic acid enhances seed germination in both the summer and winter variants significantly. However, it was relatively less effective in case of the winter variant. The effective increase in germination percentage by auxins can be explained by the fact that auxins are also responsible for inducing the synthesis of gibberellins[24].

According to Karssen[25] abscisic acid has no role in the regulation of dormancy of *C.album* seeds. From the present study we can conclude that our results in case of the winter variant are completely in agreement with Karssen[25] but the results in case of summer variant are unexplainable as the seed germination percentage increased about two to three times by the application of abscisic acid but these results do not vary significantly at different concentrations. Saini[11] reported negligible effect of kinetin on seed germination. However, in the present study kinetin was found to be a little promotive in case of the winter variant while no significant effect was observed in the summer variant.

The seeds when treated with ethephon (2-chloro ethyl phosphonic acid; an ethylene releasing compound), improved the germination in summer and winter variants when treated with 100, 500 and 1000 ppm concentrations. The results between summer and winter variants were not very significant statistically and are consistent with studies of Machabee and Saini[26]. In most of the treatments, only higher concentrations *i.e.,* 100, 500 and 1000 ppm were effective while lower concentrations were not effective.

A careful perusal of the results reveals that gibberellic acid and auxins were found to be the best among growth hormones for enhancement of seed

germination in both the variants. Ethylene was also effective equally but only in the winter variant. Kinetin has shown no significant impact. The effect of abscisic acid on summer variant remains to be an irony explained by further investigations. Another important observation is that the differences in the results of gibberellic acid and cytokinin are very highly significant. With these observations it can be concluded that effect of growth regulators in both variants of *C. album* are significantly different in their kinds and strengths.

There is very small literature available regarding the effect of temperature on the germination of *C. album*. The seeds of *C. album* germinates between the range of 2.0°C to 35°C with an optimum at 20°C[27]. For other species of *Chenopodium* like *C. glaucum* and *C. ficifolium* the optimum temperatures were 35°C and 40°C, respectively. In the present study it was observed that maximum germination in the summer variant was at 30°C while for the winter variant it was 10°C. A perusal of the data (Table 1 and 2) reveals that the seeds of summer variant have significantly higher optimum temperature for germination i.e., 30°C in comparison to winter variant which has much less optimum temperature *i.e.,* 10°C. These results are very much in agreement with the findings of Ramakrishnan and Kapoor[28]. The evidence of lower temperature optimum for the winter variant and the higher temperature requirements for the summer variant could be related to the natural temperature conditions under which they grow. Our results are further supported by statistical analysis that the differences between the variants are very highly significant at 10°C and 30°C temperatures which are also the optimum temperatures for winter and summer variants, respectively.

At higher temperatures *i.e.,* 50°C, seed germination percentage decline drastically. This may be possible because at very high temperature the enzymatic and chemical composition of seed parts including embryo are destroyed significantly which ultimately results into the loss of seed viability. Though, seeds of summer variant could resist it for some extent.

The results of this study concluded that the effect of growth regulators on seed germination in both summer and winter variants varies greatly in their kinds and strengths. However, the temperature requirement for their optimum germination is contrary to each other *i.e.,* for summer variant it is 30°C while for winter variant it is 10°C which may play important role in their ecological separation. These differences in their temperature cum photoperiod requirements for flowering[28] indicates that each population (variant) is adapted to the seasonal conditions under which they naturally grow. Therefore, they can be regarded as seasonal ecotypes because the two variants show certain constant features related to the seasons.

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