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Effects of temperature stress on Soybean seeds

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ABSTRACT

We have studied theeffects of heat treatment on the physiological and biochemical aspects in Soybean (Glycine max L) seeds.In particular, the changes ingerminability (viability), growth intensity (vigour),content and molecular size of protein,inthe seeds were investigated. The seeds were exposed at three different temperatures of 40^{0}C,50^{0}C, and60^{0}C for extended periods of 3,5, and7 days. The soybean seeds exhibited gradual reduction in viability and vigour in all the treatments.The protein content of the seeds and the molecular size of proteinsshowedreduction at lower temperature,but wereenhanced at 60^{0}C,possibly due to the phase transition of water molecules to vitrified state.

Keywords: viability, vigour, germination percentage, glassy state, vitrified state, *Glycine max*

INTRODUCTION

Plant seeds are rich in protein supplements. The members of Leguminosae family,especially the Soybean seeds, are predominantly rich in plant protein. It is well known that most plant seeds, when exposed to the higher temperature, result in the dryness of the seeds. This induces certain physiological changes in seeds manifested by the decrease in viability, vigour, protein content and molecular size of protein. Higher temperature influences depletion of free water and bound water within biomoleculesthereby causing dryness of the seeds and tighter packing of biochemicals.This in turn causes a change in structural conformation of biomolecules within seedsand results in the degradation of protein and eventual loss of seed vigour and viability.

Bewley and Black [1]demonstrated the existence of three different types of water within seeds viz. bound water i.e. adsorbed in macromolecules, free water, and vitrified state of water or glassy state of water. When bound water associated with macromolecules is lost, it results in the structural and functional deterioration of seeds. However the initial loss of free water alone, does not have significant impact on the reduction of seed potential. Presence of water as vitrified or glassy statein seeds is found in seeds when they are exposed to high temperature stressed condition (at or above 50^{0}C).At high temperature in cellular environment,the extreme low water content was found to slow downthe deteriorative chemical reactions in dry seeds [2,3].Essentially, this correspondsto a phase transition of water molecule under high temperature ambience. The low water content in the cellular environment causes the formation of amorphous thick semisolid biopolymers which startto behave in a rubber like manner, thus shielding biomolecules from direct interaction with the environmental heat stress. On the otherhand the final water content, which is extremely important for seed survival, when gradually decreases, causes not only the reduction in vigour, viability and denaturation of protein content in seeds but also results in the ultimate death of seeds.

The Soybean seeds, used in the present experiment, belong to orthodox category as they can survive temperatures as high as 60^{0}C. At such high temperature, desiccation (dryness) of soybean seeds is not observed because of their oily nature. Physiological activity i.e. vigour and viability in seeds is affected by the moisture content of the seeds and the moisture content decreases when seeds are exposed to higher temperature. As the binding strength of water within macromolecules in seeds is different, the seeds thus exhibit distinct response to temperature treatment [4,5].

The physical state of water in seeds determines the physiological manifestation that is connected with germination (viability) and intensity of growth (vigour) as well asprotein content and molecular size of protein. Ishida et al. [6] reported that most of the biological activities, including the food consumption terminates or exist at a minimum level in dry seeds.

In the present study, an emphasis has been made to observe the vigour, viability, and changes in protein content and molecular size of protein in Soybean seeds when subjected to the high temperatures (40^0C, 50^0C, 60^0C) for extended duration (3, 5, 7 days). Furthermore, an attempt has been made to determine how and to what extent the temperature treatmentresults into splitting of protein, i.e. degradation of proteins which is physiologically correlated with the phenomenon of growth and development retardation (vigour and viability) in Glycine max seedling.

MATERIALS AND METHODS

Seeds of soybean (*Glycine max L*) were used to study the correlation among the germination percentage, seedling vigour, and protein degradation. From the total available seed-lot, the seeds having uniform size, colour, and shape with intact seed coat were selected. These seeds were then subjected to temperature treatment at 40^0C, 50^0C and 60^0C for 3, 5, 7 days at each temperature. We have taken 100 seeds for each treatment. Both the temperature treated seeds as well as fresh, healthy and untreated seeds (used as control) were kept in Petri dishes and covered with lids which are also lined with moistened filter paper. The dishes were watered to avoid evaporation losses. Physiological parameters such as germination percentage, seedling vigour, protein content estimation and molecular weight determination were studied.

2.1 Germination percentage:

The germination test was terminated after 5th day and germination percentage was calculated. The emergence of radicles, each of size of 1mm, from the seeds were considered as germinated. The germination percentage was calculated using the following formula:

$$Germination\ (\%) = \frac{Number\ of\ seeds\ germinated}{Total\ Number\ of\ seeds\ taken\ for\ germination\ studies} \times 100$$

2.2 Seedling vigour:

The vigour of seedlings for the control and temperature treated soybean seeds was calculated by measuring the total seedling(root-shoot) length. For vigour study, germination plate was terminated after 5 days and germination percentage and total seedling length were recorded for testing the vigour and viability of seeds.

2.3 Protein estimation by Lowry's method:

The samples of Glycine max (soybean) seeds that are physiologically uniform in size and shape were selected and used as experimental material for the present work. Crude protein content of treated and control seeds (volume of control and treated sample taken 0.1ml) was estimated following the standard method suggested by Lowry et al (1951).

2.4 Polyacrylamide- Sodium Dodecyl Sulphate slab Gel electrophoresis (SDS-PAGE) of proteins

Electrophoresis is widely used to separate and characterize proteins by applying electric current. Electrophoretic procedures using Sodium Dodecyl Sulphate (SDS) containing polyacrylamide gel is relatively sensitive and rapid requiring only micro weights of proteins (25 microgram of sample taken for each case). We have employed this method to determine the molecular weight of soybean protein where globulin and its subunits exhibited as major bands in the acrylamide gel slab.

RESULTS AND DISCUSSION

The seeds of Soybean (Glycine max L) exhibited about 77% germination before they were exposed to temperature treatment. When the seeds were treated at 40^0C, 50^0C, and 60^0C then the viability and vigour declined drastically (Table 1). At 40^0C and with varying days (3, 5, 7), the seed viability decreased successively by ~10% and the vigour also showed significant reduction as compared to control. As compared to the control, significant variations in the number of Soybean seeds that have germinatedwith increasing number of days and at different temperatures, were found. Although the viability declined steadily with both the increasing temperature and days, butthe seeds treated at 40^0C for 3 days maintained about 60% germination (Fig. 1) which may be due to the loss of free water alone whereas the adsorbed water within the macromolecules remains persistent.

Table 1:Viability, Vigour, Protein content, and Molecular weight of protein in temperature treated seed samples of *Glycine max* (Soybean)

Treatment	Days	Viability %	Vigour (cm)	Protein content μg/0.1 *ml*	Molecular weight of protein band (*KDa*)		
Control	3	76	12.2	106	70	45	20
	5	80	12.9	104	-	-	-
	7	74	12.1	102	-	-	-
40^0C	3	64	6.83	76	70	44	20
	5	56	6.76	96	70	41	-
	7	45	6.53	28	68	41	-
50^0C	3	41	5.87	86	68	31	-
	5	36	5.03	72	65	30	-
	7	32	4.63	38	64	-	-
60^0C	3	53	6.03	100	65	29	-
	5	26	4.27	110	67	-	-
	7	14	2.15	134	70	30	-

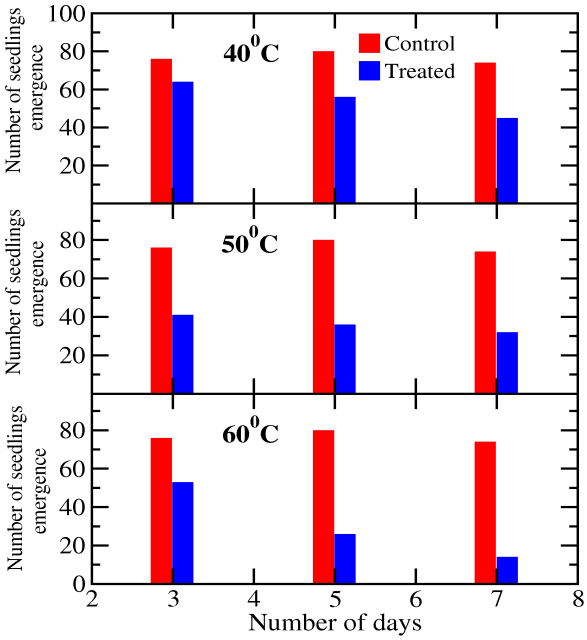

Figure 1:Number of germinated seedling of *Glycine max* (Soybean) with increasing days at temperatures of 40^0, 50^0 and 60^0C

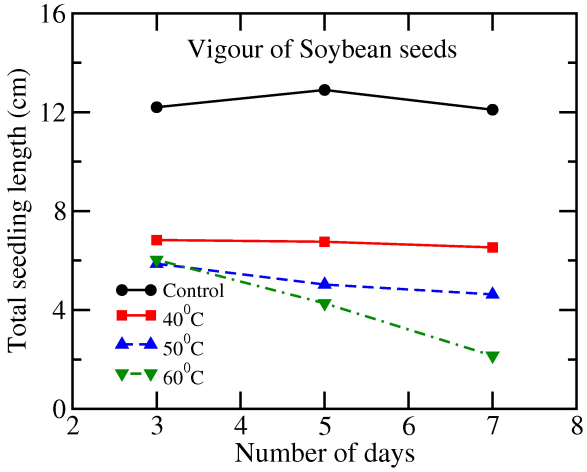

Figure 2:Seedling length in *Glycine max* (Soybean) subjected to temperature stress condition at 40^0, 50^0 and 60^0C

On the other hand, heat treatment at 60^0C displayed reduction in viability and vigour when the seeds were exposed for extended duration (3, 5, 7 days). Even the molecular weight of protein bands was found to have decreasing weight in compare with control (Table 1). However, some of these protein bands reappeared those were otherwise lost at 40^0C and 50^0C. Moreover, the protein content at 60^0C is in increasing order as compared to the seed samples treated at 40^0C and 50^0C. One explanation could be, that the seeds, struggle to survive by shielding the molecular

denaturation of protein by glass formation or vitrification of water thereby protecting the protein content from direct interaction with the temperature stress [1].

Correlation between the varying number of days and the seedling length at different temperaturescan be established. Seedssubjected to drying at high temperature shows significant loss of vigour(Table 1), possiblydue to conformational change and hence molecular destruction of biochemicalswithin seeds at all the treated temperatures and varying days. It may be presumed that during the temperature treatment, the tissues lose water molecules that are adsorbed in the macromolecules of the cell membranes along with free water. This supportsthe findings of Bewley and Black [1], who suggested that the adsorbed water or bound water is loosely held by bonding to macromolecules.At 40^0C, the considerable germinability(as observed in Figs. 1-2) indicates the metabolic activities of the seed remain almost normal even in the absence of free water. Above 40^0C, the rate of viability loss was observed to be gradual and more or less continuous. At temperature as high as 60^0C, it seems that significant amount of bound water within macromolecules is removedas viability is found to be 14% (Table 1, Fig. 2).

Various studies on seed vigour and viability confirm that ambient temperature is one of the major important factors in the maintenance of seed vigour and viability. Within a certain limit, lower the seed moisture and temperature, the longer is the viability maintained [7]. Moreover, different factors have been found responsible for the loss of viability: (i) Changes in the seed reserves; like carbohydrates, proteins, lipids, (ii) Enzymatic and metabolic activities [8,9], (iii) Changes in membrane integrity [10], (iv) Breakage of chromosomes [11], and (v) Accumulation of automutagenic substances [12] during the early hours of imbibition.

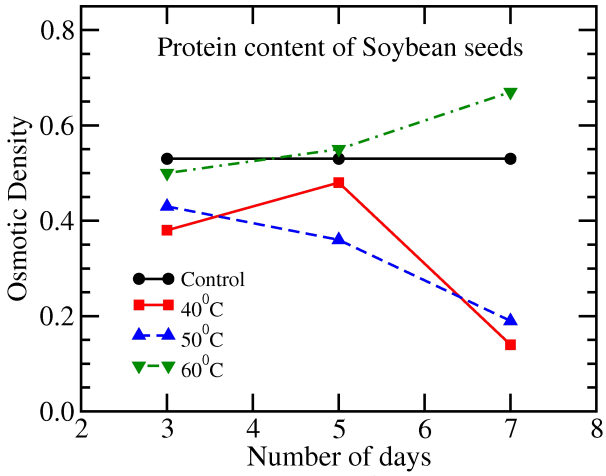

Figure 3:Osmotic density for protein in temperature treated seed samples of *Glycine max* (Soybean) with the increase in days at different temperatures

Variations in the osmotic density (OD) of the protein content in*Glycine max* (Soybean) seeds with increasing number of days and temperatures is presented in Fig. 3. At temperatures below 60^0C, the OD values tend to be reduced due to the loss of moisture. Whereas, at the maximum temperature of 60^0C studied here, the OD values shows a steady increase which could be explained as due to the presence of glassy transition of water in the seeds [1]. This glassification stateslows down and finally stops chemical interaction of biomolecules to heat stress, thus assures stability and quiescence of cell chemical components. In-vitro studies have shown that vitrification or glassy state of water retards or preventsthe denaturation of proteins including enzymes [13]. Thus the present study may suggest that even the vitrified water in Soybean seeds is protecting the protein content in such a way that it remains unavailable to the temperature stress and is not subjected to denaturation at 60^0C (Table 1, Fig. 3) but results in gradual loss of viability and vigour.

An alternative explanation for the viability of seeds being retained at high temperatures like 60^0C may be the maintenance of vitrification or glass formation of water within the cytoplasm, which is the potential mechanism to avoid the mobility of nutrients and thus slowing down the deleterious activity of biomolecules pertaining to seed destruction, crystallization of proteins and solutes present in the cytoplasm. The major function of the glassy state of water in the dry seeds is its contribution to the stability of the seed components during storage and thus to the survival during desiccation [3].

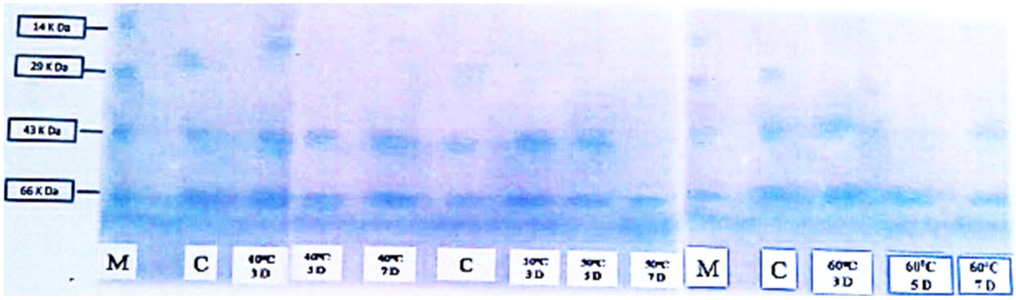

Figure 4:SDS electrophoresis of protein extracted from seed samples of *Glycine max* subjected to the temperature stress condition

The studies established on SDS-PAGE electrophoretic band pattern with known standard marker illustrated the presence of an important protein component of seed i.e. Globulin. This is the major protein component in the leguminous seed (soybean) which has been analysed and studied in details in various leguminous members. This molecule has two subunits like 7S and 11S. Utsumi and Kinsella [14] have investigated the gelatin of globulin in soybean and reported that 7S subunit of globulin predominantly has hydrogen bonding, and 11S subunit has disulphide and hydrogen bonding. They reported the presence of 2-3 Cysteine groups per molecule of globulin subunits (7S and 11S); the latter constitute about 87% of total proteins. The 7S globulin in soybean has 6 molecular isomers. The major bands evident in slab gel are subunits of 11S Globulin protein. The 11S Globulin subunit was present in its reduced subunit form (Table 1).

The protein bands with molecular size alongwith molecular marker are shown in Fig. 4. For the observed four bands, the R_F values for standard protein marker are 0.39, 0.52, 0.62, 0.79 with molecular weight 66, 43, 29, and 14 *kDa*, respectively. For the untreated (control) seeds, the $R_F$values from the lowest to highest bands were observed to be 0.83, 0.66 and 29. These correspond to R_F values of the marker proteins to be 0.79, 0.62 and 0.52, respectively. Thus the molecular weight of the lowest band of untreated seeds is approximately 70 *kDa*, and the highest band's molecular weight is 29 *kDa*. Similarly, it was observed that the bands of treated Soybean seeds at 40^0C for 3 days have $R_F$values of 0.83, 0.66, 0.52 which corresponds to the molecular weights of proteins to be 70, 44, 20 *kDa* with reference to the molecular marker. Again, Soybean sample treated at 40^0C for 5 and 7 days shows bands of molecular weights of approximately 68-70 *kDa* for the lowest band and 41 *kDa* for the higher bands. More importantly, the 3rd top most bands have disappeared. This indicates the denaturation of protein in the treated seedsat the temperature of 40^0C for longer duration treatment. Soybean sample treated at 50^0C for 3 and 5 days shows the existence of 2 bands in each case having the molecular weight ranging 68-65 *kDa* for lower bands and 30-31 *kDa* for higher bands. On the other hand, at 50^0C, heat treatment for 7 days shows the presence of single band understating the denaturation of other protein bands at such high temperature. Furthermore, Soybean samples treated at 60^0C for 3 days and 7 days show the reappearance of protein bands with the molecular weight ranging from 65-70 *kDa* for the lower bands and 29-30 *kDa* for the topmost bands. This observation indicatesthe presence of glassy state of water at high temperature of 60^0C thus protecting protein molecules from direct interaction with the induced temperature stress.

CONCLUSION

In the present study,we have exposed Soybeanseedsat various temperatures to determine the changes in vigour, viability, content and molecular weight of denaturing protein.Our findings indicatethat the loss of germination percentage (viability) is succeeded by loss of vigour when the seeds are eventually exposed to the increasingtemperatures of 40^0C, 50^0C, 60^0C for extended period (3-7days). The seeds treated at 40^0 and 50^0C for 3, 5, 7 daysexhibit the reduction in the content of protein compared to untreated seeds. This establishes the fact that excessive heat causes the degradation of protein.
In contrast, the protein content of the treated seeds does not show any reduction at 60^0C.This indicates that vitrification of water prevents the breakdown of proteins. Moreover, this suggests that the glass formation of water within the cytoplasm is the potential mechanism to avoid the crystallization and denaturation of protein.

Banding pattern of untreated seeds is found similar to treated sample at 40^0Cfor 3 days. However, with the increase of days (i.e. 5-7 days), the number of bands decreases and appearance became much lighter which suggests the denaturation of protein in Soybean seeds at this temperature. Similar banding pattern has been observed in case of Soybean sample treated at 50^0C for 3-7 days. However, these protein bands reappear when the seeds were treated at 60^0C for 3-7 days. This emphasizes the fact that the vitrification of water retards or prevents denaturation of proteins at this high temperature stress.

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