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Estimation of Hormonal Seed Treatments on Enzyme Activities after Accelerated Ageing (Artificial Ageing) in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Background: Accelerated ageing, which is known to diminish seed viability and vigour in different seed crops, has been used to predict seed storability. The goal of this study was to see how accelerated ageing affected enzymatic characters of chickpea seeds (Variety Pusa 362). Seeds were exposed to accelerated ageing conditions by being heated to 35°C and 40°C and kept at 90% and 100% relative humidity at different duration *i.e.*, 2, 4 and 8 days. After accelerating ageing pre sowing seed treatment with salicylic acid and gibberellic acid has done. The findings revealed that faster ageing causes the chickpea seed to degenerate, which is linked to a decline in enzymatic characters but hormonal seed treatments enhances enzymatic (amylase, catalase, peroxidase, dehydrogenase and protease) performance after ageing. Our result showed that antioxidant activity of aged seeds increased after seed treatment with plant growth hormones.

Methods: The present experiment was conducted at state seed testing laboratory, Department of Genetics and Plant Breeding, SHUATS, Prayagraj, Uttar Pradesh during the year 2018 to 2020. In the experiment treatment were comprises with 37 treatment combination. The seeds are exposed to high temperatures and relative humidity in a chamber to speed up the ageing process. After that aged seed were invigorated with hormones *i.e.*, hormonal seed priming.

Result: Our finding revealed that the aged seed treated with gibberellic acid and salicylic acid enhanced the activity of antioxidant enzymes which help in seed germination.

Key words: Chickpea, Gibberellic acid, Hormonal seed treatment, Salicylic acid, Seed ageing.

INTRODUCTION

Chickpea is a self-pollinated genuine diploid (2n = 2 x = 16) that belongs to the Fabaceae family. It is an ancient human-cultivated cool-season food legume crop that has been discovered in Middle Eastern archaeological sites dating from 7500-6800 BC (Zohary and Hopf, 2000). Chickpea is the second most critical food vegetable later essential bean (FAOSTAT, 2011). Chickpea is known to have begun in western Asia (presumably eastern Turkey). Pulses have traditionally been deemed “the poor man’s meat” since they are one of the least expensive sources of protein in Indian farming and consumption patterns (Mohanty and Satyasai, 2015). Chickpea (*Cicer arietinum*) is chosen above food legumes among pulses because of its various uses for the world’s rising population. It was grown on 149.66 lakh ha in 2017-18, with a total production of 162.25 lakh tonnes and an average productivity of 1252 kg/ha (FAOSTAT, 2019). Plants with well-developed root systems are more able to withstand adverse conditions. Seed ageing is the most serious problem with seed storage. In improper storage circumstances with high temperature and moisture, seed vigour and viability are diminished (Sveinsdottir *et al.*, 2009).

Seed deterioration is loss of seed quality, viability and vigour due to effect of adverse environmental factors (Kapoor *et al.*, 2010). Deteriorative changes enhance when seed exposure to external challenges which decreases the ability of the seed to survive. Annual losses due to deterioration can be as much as 25% of the harvested pulses crop. It is one of the basic reasons for low productivity (Shelar *et al.*, 2008). The process has been described as cumulative, irreversible, degenerative and inexorable process (Kapoor *et al.*, 2011). As seed deterioration increases, seed performance progressively decreases. Losses of seed quality occur during seed production, harvesting and storage. Several factors contribute to the susceptibility for seed deterioration. The basic causes are occurred through temperature, relative humidity, seed moisture content and by invasion of microorganisms and insects. The accelerated

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ageing technique is a frequently used tool for assessing seed quality, according to Pandey *et al*., (1990). This ageing test of seed vigour, rather than germination and growth tests, can provide more accurate estimates of probable field emergence for vegetable crop seeds. Accelerated ageing procedures provide a lot of promise for research into seed ageing mechanisms and deterioration processes (McDonald, 1999). During seed germination, specific enzymes must be triggered at specific times. The oxidative phosphorylation pathway is activated when seeds improve their oxygen uptake (Tommasi *et al.,* 1999). Oxidative phosphorylation and the mobilization of food storage produce reactive oxygen species (ROS), which can impair the structural and functional makeup of cells. As a result, germination is assumed to be dependent on the enzymes that scavenge ROS. Because this scavenging process might affect seed storage and vigour, the efficiency of free radical scavenging seeds may be linked to the percentage of seed germination (Bailly *et al.,* 1996). Seed quality is the most important determinant of stand establishment in any crop and it is therefore of paramount relevance and priority in the case of high volume, low value crops in general for increased productivity and production.

Therefore, findings demonstrated that as the chickpea seed ages, it degenerates, which is associated to a loss in enzymatic characteristics, but hormonal seed treatments improve enzymatic performance (amylase, catalase, peroxidase, dehydrogenase and protease) after ageing. In this study, we show how accelerated ageing causes a significant fall in enzymatic measures and seed fortification enhance the antioxidant enzymes measures under accelerated ageing (Artificial ageing) in chickpea seeds.

MATERIALS AND METHODS

The experiment was carried out in the state seed testing laboratory of Seed Science and Technology at Department of Genetics and Plant Breeding, SHUATS, Prayagraj, Uttar Pradesh during 2018-20.The state seed testing laboratory, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Science, Naini Agricultural Institute, Prayagraj, provided chickpea seeds of variety (Pusa) (U.P.). The experimental material comprised with 37 treatments (with control). For the accelerated ageing treatments, seeds were cultured in airtight plastic boxes. Seeds were placed at 35°C and 40°C with a relative humidity (RH) of 90 and 100 per cent for 2, 4 and 8 days in three different accelerated ageing regimes prescribed by (Delouche and Baskin, 1973). Then, aged chickpea seeds were invigorated in a hormonal solution of Salicylic acid Gibberellic acid *i.e*., hormonal seed fortification (Afzal *et al.,* 2006). For making solution, 100 mg of every substance were taken in a container. These synthetics were included 1000 ml. of distilled water with steady blending. The volume of arrangement was at long last comprise to one litter and then it became 100 ppm stock arrangement of every compound. After 12 hr of soaking the arrangement was emptied out of the measuring beaker and pre-soaked seed had air dried to conduct enzymatic extraction. Total Amylase (α amylase E.C. 3.2.1.1 and β amylase E.C. 3.2.1.2) was measured colorimetrically using the Sumner and Howell 1935 protocol, which involved measuring the amount of maltose liberated from starch. The peroxidase activity (E.C.1.11.1.7) was assayed to the method prescribed by (*Rao et al*., 1996). The catalase enzyme (E.C. 1.11.1.1) was assayed according to the method of Sinha 1972. The protease activity (E.C. 3.4.21.112) was assayed according to Issac and Gokhale (1982) and dehydrogenase activity (E.C. 1.1.1) assayed to the method prescribed by Kittock and Law (1968).

The analysis of variance was worked out to test the significant differences among treatments by F- test. It was carried out according to the procedure of complete block design foreach character as per methodology suggested by Fisher (1936).

RESULTS AND DISCUSSION

Among the accelerated ageing durationan activity of all enzymes in each treatment fluctuated extensively throughout the trial. The higher activity of all the enzymes was recorded in seeds treated with T_{13} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (2)] followed by T_{14} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (4)] which were on par with T_{26} [Salicylic Acid +RH (90%) +Temp. (35°C) +Days (4)] and T_{31} [Salicylic Acid +RH (100%) +Temp. (35°C) +Days (2)] while significantly lowest activity of all the enzymes was recorded in T_{12} [RH (100%)+Temp. (40°C)+Days (8)] under accelerated ageing. These results are parallel to those of the studies (Kaur *et al.,* 2005) GA_3 modulates the activity of many enzymes, particularly amylase and increases the mobilization of starch granules in cotyledons, promoting germination and growth (Yadollhhi and Mashayekhi 2013). Seed deterioration due to ageing is a natural and inexorable phenomenon which is regulated by various metabolic activities especially related to protein and lipid metabolism as well as the generation of free radicals and antioxidant system present in the seed (Khan *et al.,* 2016). Decreasing of germination percentage in aged seeds can be due to reduction of α-amylase activity and carbohydrate contents (Bailly, 2002), or denaturation of proteins (Nautiyal *et al.,* 1985). Priming increases seed reserves utilization under unfavorable conditions there for priming by increased these traits can be improved germination characteristics under aging and correlation with antioxidant enzymes activity.

The activity of α-amylase was recorded higher in seeds treated with T_{13} [Gibberellic acid + RH (90%) + Temp. (35°C) + Days (2)] (0.872 µmole/mg) followed by T_{14} [Gibberellic acid + RH (90%) + Temp. (35°C) + Days (4)] (0.836 µmole/mg), which were on par with T_{26} [Salicylic acid +RH (90%) + Temp. (35°C) +Days (4)] (0.795 µmole/mg) and T_{31} [Salicylic Acid +RH (100%) +Temp. (35°C) + Days (2)] (0.789 µmole/mg) while significantly lower activity of α-amylase was recorded

in T_{12} [RH (100%) +Temp. (40°C) +Days (8)] (0.308 µmole/mg). Similarly, The higher activity of α amylase was recorded in seeds treated with T_{13} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (2)] (0.741 µmole/mg) followed by T_{14} [Gibberellic acid + RH (90%) + Temp. (35°C) +Days (4)] (0.734 µmole/mg), which were on par with T_{25} [Salicylic acid + RH (90%) +Temp. (35°C) + Days (2)] (0.718 µmole/mg) and T_{26} [Salicylic acid +RH (90%) + Temp. (35°C) +Days (4)] (0.703 µmole/mg), while significantly lower activity of β-amylase was recorded in T_{12} [RH (100%) +Temp. (40°C) +Days (8)] (0.416 µmole/mg). Our results showed that the total amylase activity declined to increase with the ageing duration but seed invigoration with gibberellic acid and salicylic acid in aged seed increased the activity of total amylase (α-amylase and β-amylase).

The hormonal treatment with Gibberellic acid at RH (90%) and temperature (35°C) for 2 days ageing showed minimum activity of peroxidase, whereas peroxidase activity lowers gradually with the increase in relative humidity, temperature and duration of accelerated aging. The higher activity of peroxidase was recorded in seeds treated with T_{13} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (2)] (0.495 µmole/mg) followed by T_{14} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (4)] (0.471 µmole/mg), which were on par with T_{25} [Salicylic Acid +RH (90%) +Temp. (35°C) +Days (2)] (0.463 µmole/mg) and T_{26} [Salicylic Acid +RH (90%) +Temp. (35°C) +Days (4)] (0.428 µmole/mg), while significantly lower activity of peroxidase was recorded in T_0 [RH (100%) +Temp. (40°C) +Days (8)] (0.275 µmole/mg). Our results showed that catalase and peroxidase activity was reduced by increment of period of aging. Therefore, priming significantly improved studied enzymes activity. These results are parallel to those of the Seiadat *et al.* (2012), Ghassemi-Golezani *et al.* (2012), Ansari and Sharif Zadeh (2013) and Sedghi *et al.* (2010). Bailly *et al.* (1996) reported that a decrease in antioxidant enzymes is linked to an increased lipid peroxidation and accelerated ageing. The impact of priming is dependent on the variety, seed age and treatments used. As a result, conclude that there is no universal use of a single priming, as it may not be appropriate for each cultivar and may result in a reduction in germination energy and germination.

The higher activity of catalase was recorded in seeds treated with T_{13} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (2)] (0.544 µmole/mg) followed by T_{14} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (4)] (0.527 µmole/mg), which were on par with T_{25} [Salicylic Acid +RH (90%) +Temp. (35°C) +Days (2)] (0.506 µmole/mg) and T_{15} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (8)] (0.509 µmole/mg), while significantly lower activity of catalase was recorded in T_{12} [RH (100%) +Temp. (40°C) +Days (8)] (0.336 µmole/mg). The higher activity of protease was recorded in seeds treated with T_{13} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (2)] (1.396 µmole/mg) followed by T_{14} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (4)] (1.333 µmole/mg), which were on par with T_{25} [Salicylic Acid +RH (90%) +Temp. (35°C) +Days (2)] (1.296 µmole/mg) and T_{26} [Salicylic acid +RH (90%) +Temp. (35°C) +Days (4)] (1.221 µmole/mg), while significantly lower activity of protease was recorded in T_{12} [(RH (100%) +Temp. (40°C) +Days (8)] (0.779 µmole/mg). The higher activity of dehydrogenase was recorded in seeds treated with T_{13} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (2)] (0.491 µmole/mg) followed by T_{14} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (4)] (0.479 µmole/mg), which were on par with T_{25} [Salicylic Acid +RH (90%) +Temp. (35°C) +Days (2)] (0.462 µmole/mg) and T_{27} [Salicylic Acid +RH (90%) +Temp. (35°C) +Days (8)] (0.456 µmole/mg), while significantly lower activity of dehydrogenase was recorded in T_{12} [(RH (100%) +Temp. (40°C) +Days (8)] (0.220 µmole/mg). In various crops seed priming treatments and post priming treatments have been utilized to reduce ageing damage and improve performance (Basra *et al.*, 2003; Farooq *et al.*, 2006; Ansari *et al.*, 2013). Priming improves germination qualities during ageing and correlates with antioxidant enzyme activity by increasing seed reserves consumption under unfavorable conditions According to Abdalla and Roberts (1968). It has been reported that in aged seeds, antioxidant enzyme activity such as superoxide dismutase, catalase, peroxidase and glutathione reductase decreases. This decrease in enzyme activity lowers the seed's respiratory capacity, lowering both the energy (ATP) and assimilates supply of the germinating seed (McDonough, 2004). Khajeh *et al.*, (2015) suggested that seed ageing is associated with a decrease in enzyme activity, which may contribute to low seed germination efficiency; priming, on the other hand, enhances enzyme activity, which may lead to improved germination characteristics. The general decrease in enzyme activity in the seed reduces the seed's respiratory capacity, which reduces the sprouting seed's energy (ATP) and assimilates supply. Our findings show that priming can enhance total amylase, catalase protease, peroxidase and dehydrogenase activity in aged seeds. Invigorated seeds indicated that highest dehydrogenase activity (OD 10 min^{-1}),catalase activity, peroxidase activity (OD 10 min^{-1}) (µg H_2O_2 $mg^{-1}min^{-1}$) under accelerated aged seeds of chickpea (Hridya *et al.*, 2018). Ageing methods had significant negative effect on seed physiological and biochemical quality parameters. Catalase activity was dramatically reduced in accelerated ageing as a result of greater temperature and relative humidity (Patil *et al.*, 2021).

As shown in Fig 1 and Table 1, the average performance of α-amylase, β-amylase, peroxidase activity, catalase activity, protease activity and dehydrogenase due to the effects of hormonal seed treatment after accelerated aging in chickpea (*Cicer arietinum* L.)

Table 2 gives the association of maltose at various concentrations with the best five treatments of total amylase and Fig 2 best. Histogram showing the association of maltose at different concentrations with Five treatments of total amylase.

Table 1: Mean performance of α-amylase, β-amylase, peroxidase activity, catalase activity, protease activity and dehydrogenase due to the influence of hormonal seed treatments after accelerated ageing in chickpea (*Cicer arietinum* L.).

Tr. no	Treatments	Alpha amylase activity	Beta amylase activity	Peroxidase activity	Catalase activity	Protease activity	Dehydrogenase
T_0	Control (Untreated)	0.624	0.592	0.332	0.420	0.894	0.386
T_1	RH (90%)+Temp. (35°C)+Days (2)	0.595	0.512	0.302	0.389	0.862	0.346
T_2	RH (90%)+Temp. (35°C)+Days (4)	0.543	0.505	0.290	0.385	0.855	0.340
T_3	RH (90%)+Temp. (35°C)+Days (8)	0.552	0.504	0.298	0.383	0.870	0.333
T_4	RH (90%)+Temp. (40°C)+Days (2)	0.500	0.520	0.294	0.380	0.870	0.358
T_5	RH (90%)+Temp. (40°C)+Days (4)	0.440	0.506	0.296	0.378	0.849	0.332
T_6	RH (90%)+Temp. (40°C)+Days (8)	0.525	0.526	0.320	0.434	0.964	0.358
T_7	RH (100%)+Temp. (35°C)+Days (2)	0.486	0.515	0.299	0.388	0.891	0.336
T_8	RH (100%)+Temp. (35°C)+Days (4)	0.407	0.512	0.277	0.382	0.875	0.327
T_9	RH (100%)+Temp. (35°C)+Days (8)	0.394	0.521	0.290	0.391	0.866	0.334
T_{10}	RH (100%)+Temp. (40°C)+Days (2)	0.356	0.515	0.301	0.379	0.870	0.343
T_{11}	RH (100%)+Temp. (40°C)+Days (4)	0.602	0.440	0.315	0.379	0.838	0.319
T_{12}	RH (100%)+Temp. (40°C)+Days (8)	0.308	0.416	0.275	0.336	0.779	0.220
T_{13}	Gibberellic acid+RH (90%)+Temp. (35°C)+Days (2)	0.872	0.741	0.495	0.544	1.396	0.491
T_{14}	Gibberellic acid+RH (90%)+Temp. (35°C)+Days (4)	0.836	0.734	0.471	0.527	1.333	0.479
T_{15}	Gibberellic acid+RH (90%)+Temp. (35°C)+Days (8)	0.665	0.589	0.401	0.509	1.271	0.409
T_{16}	Gibberellic acid+RH (90%)+Temp. (40°C)+Days (2)	0.746	0.669	0.385	0.483	1.228	0.379
T_{17}	Gibberellic acid+RH (90%)+Temp. (40°C)+Days (4)	0.645	0.646	0.368	0.460	1.189	0.363
T_{18}	Gibberellic acid+RH (90%)+Temp. (40°C)+Days (8)	0.590	0.607	0.340	0.442	1.114	0.317
T_{19}	Gibberellic acid+RH (100%)+Temp. (35°C)+Days (2)	0.761	0.694	0.404	0.443	1.201	0.326
T_{20}	Gibberellic acid+RH (100%)+Temp. (35°C)+Days (4)	0.567	0.565	0.376	0.430	1.176	0.298
T_{21}	Gibberellic acid+RH (100%)+Temp. (35°C)+Days (8)	0.475	0.530	0.366	0.403	1.154	0.271
T_{22}	Gibberellic acid+RH (100%)+Temp. (40°C)+Days (2)	0.507	0.495	0.351	0.379	1.147	0.242
T_{23}	Gibberellic acid+RH (100%)+Temp. (40°C)+Days (4)	0.419	0.458	0.335	0.362	1.075	0.229
T_{24}	Gibberellic acid+RH (100%)+Temp. (40°C)+Days (8)	0.356	0.537	0.307	0.388	0.987	0.352
T_{25}	Salicylic acid+RH (90%)+Temp. (35°C)+Days (2)	0.751	0.718	0.463	0.506	1.296	0.462
T_{26}	Salicylic acid+RH (90%)+Temp. (35°C)+Days (4)	0.795	0.703	0.428	0.495	1.221	0.439
T_{27}	Salicylic acid+RH (90%)+Temp. (35°C)+Days (8)	0.712	0.670	0.451	0.483	1.164	0.456
T_{28}	Salicylic Acid +RH (90%)+Temp. (40°C)+Days (2)	0.756	0.652	0.425	0.469	1.156	0.432
T_{29}	Salicylic Acid +RH (90%)+Temp. (40°C)+Days (4)	0.664	0.621	0.408	0.457	1.133	0.416
T_{30}	Salicylic Acid +RH (90%)+Temp. (40°C)+Days (8)	0.596	0.596	0.392	0.444	1.036	0.379
T_{31}	Salicylic Acid +RH (100%)+Temp. (35°C)+Days (2)	0.789	0.613	0.400	0.458	1.202	0.419
T_{32}	Salicylic Acid +RH (100%)+Temp. (35°C)+Days (4)	0.619	0.575	0.385	0.442	1.171	0.396
T_{33}	Salicylic Acid +RH (100%)+Temp. (35°C)+Days (8)	0.505	0.560	0.351	0.424	1.131	0.363
T_{34}	Salicylic Acid +RH (100%)+Temp. (40°C)+Days (2)	0.518	0.530	0.348	0.419	1.032	0.329
T_{35}	Salicylic Acid +RH (100%)+Temp. (40°C)+Days (4)	0.463	0.502	0.321	0.404	0.967	0.305
T_{36}	Salicylic Acid +RH (100%)+Temp. (40°C)+Days (8)	0.418	0.469	0.294	0.383	0.919	0.276
Grand mean		0.571	0.572	0.569	0.426	0.779	0.355
CD at level of 5% significance		0.007	0.008	0.009	0.115	0.105	0.105
Range	Max	0.872	0.741	0.495	0.544	1.396	0.491
	Min	0.308	0.416	0.275	0.336	0.779	0.220

Table 2: Collaboration of maltose at different concentrations with best five treatments of total amylase.

Maltose concentrations	1st readings (Maltose)	α-amylase (Treatments)	2nd readings (Maltose)	β-amylase (Treatments)
(0.5 mg)	1.082	0.872 (T_{13})	0.951	0.741 (T_{13})
(0.8 mg)	0.961	0.751 (T_{25})	0.928	0.718 (T_{25})
(1.10 mg)	1.005	0.795 (T_{26})	0.913	0.703 (T_{26})
(1.40 mg)	0.971	0.761 (T_{19})	0.904	0.694 (T_{19})

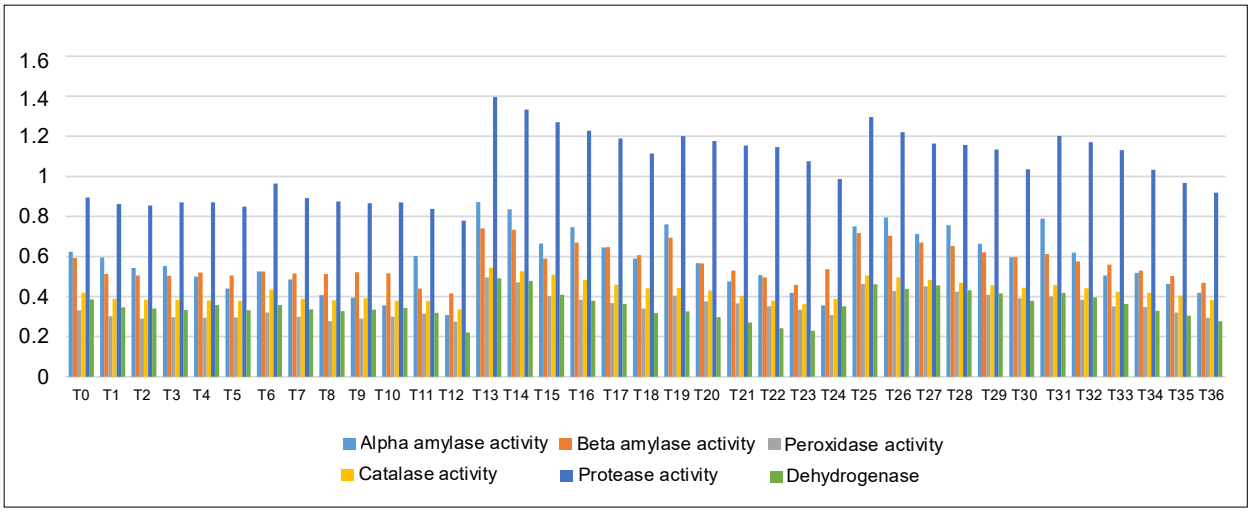

Fig 1: Histogram depicting mean performance of total amylase activity (α and β amylase), peroxidase activity, catalase activity, protease activity and dehydrogenase activity due the influence of hormonal seed treatments after accelerated ageing in chickpea (*Cicer arietinum* L.).

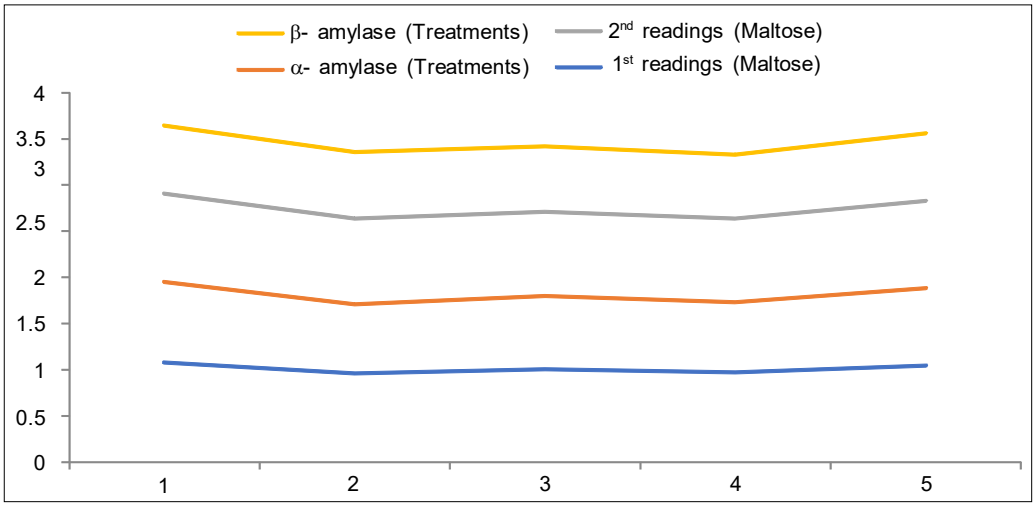

Fig 2: Histogram depicting collaboration of maltose at different concentrations with best five treatments of total amylase.

CONCLUSION

From present experiment it is concluded that the humidity, temperature and the length of time seeds are exposed to ageing conditions can all have an impact on seed quality. With increase in duration of ageing highly decreased seedling growth enzymes. Seed treatment with growth hormone in aged seeds increases the activity of enzymes. The higher activity of all enzymes was recorded in seeds treated with T_{13} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (2)] followed by T_{14} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (4)], which were on par with T_{25} [Salicylic Acid +RH (90%) +Temp. (35°C) +Days (2)] and T_{15} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (8)], while significantly lowest activities of enzymes was recorded in T_{12} [RH (100%) +Temp. (40°C) +Days (8)]. Capacity of invigorated seeds to scavenge free radicals by elevated

enzymes catalase and rapid mobilization of stored carbohydrates and proteins by amylase and proteases during germination could at least partially explain the beneficial effects of invigoration. Gibberellic acid (GA3) and salicylic acid (SA) can contribute in mitigation of deleterious effects of stress and can improve seed germination percentage. Therefore, accelerated ageing test can predict the storage potential and longevity of seeds.

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Conflict of interest

Authors declare no conflict of interest.

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