

THERMAL HARDENING IMPROVES GERMINATION AND EARLY SEEDLING GROWTH OF CHICKPEA

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ABSTRACT

Poor quality seeds of chickpea have germination problem and issue is serious in chickpea growing areas where quality seed is not available. This study was an attempt to solve the problem. Experiment was done in Seed Technology Laboratory of Department of Agronomy, University of Agriculture, Faisalabad. Two chickpea cultivars viz. Kabli type cultivar Noor-91 and desi type cultivar Punjab-91 were treated with different temperatures of 40, 50 and 60 °C at 24, 48 and 72 hrs. Different thermal hardening treatments significantly improved different germination related traits including germination percentage, germination index, germination energy and reduced time taken for 50% germination. Similarly heating of chickpea seeds resulted in more number of secondary roots, and improved fresh and dry weights. Although germination was improved with thermal treatments at 40 and 50 °C for different time durations, however, an increased temperature (60 °C) was better only at 24 hrs and keeping seeds at this temperature for more time resulted in reduced germination and other traits. It is thus concluded that thermal hardening at 40 and 50 °C for up to 72 hrs can be used to improve germination of chickpea.

Keywords: Chickpea, Germination Index, Germination Energy, Dry weight, Temperature.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a chief protein rich source of cool-season usually grown under rainfed environments of the world. It is an important human consumption which is roughly alienated into desi with dark brown coat (Microsperma) and kabli with light yellow covering (Macrosperma) (Muehlbauer and Singh, 1987). Both are used as different food stuff due to difference in seed size, color, water uptake and cooking properties, crude fiber and caloric value (Khan et al., 1995). As grown mostly under rainfed environments, a good stand is possible in good dew precipitation.

Seed hardening is a technique which has been used successfully for vigor enhancement in several seeds like rice, wheat (Basra et al., 2003, 2004; Lee and Kim, 2000). In this technique, seeds may be exposed to different conditions like alternate wetting and drying, chilling treatment or high temperature for different durations (Farooq et al., 2005). The practice of heating, chilling or hydration may be repeated several times according to the seed structure or composition. (Lee and Kim,

2000). It is possible to carry out thermal treatments using dry heat by keeping seeds in an oven at varying controlled temperatures with a series of temperatures imitating the conditions that may exist in the soil with different exposure times (Perez and Pita, 1997).

Several researchers worked on hardening and other techniques for increase in vigor of seeds and found hardening better than other. For example, in a study on rice seeds, Farooq et al. (2004) exposed seeds to dry heat (at 40 °C for 72 h, at 60 °C for 24 h) and chilling (at -19 °C for 72 h). The seeds treated with dry heat at 40 °C for 72 h found with more vigor as compared to chilling treatments. Earlier, Lee and Kim (2000) compared osmoconditioning and hardening for germination of standard and physically aged seeds with analysis of α -amylase activity and total sugars. Although total sugar content and α -amylase activity increased with both techniques but hardening was found to be more effective than osmoconditioning.

Many plant species in different climatic regions of the world are exposed to the passage of fire and it is found that rather a

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destructive influence, it triggers and facilitates in germination and seedling establishment (Whelan, 1995). Seeds of such species not only tolerate high temperatures more than 100 °C, but also frequently respond with high germination rate following the heat shock. It is argued that these seeds may have a dormancy imposed by an impervious seed coat (Cavanagh, 1987) or alterations to cell structure of seed (Tran and Cavanagh, 1980). The exposure to high temperature and heat shock to such seeds is considered to scarify the seed coat and allow water and gas exchange resulting in smashed seed dormancy (Thanos and Georghiou, 1988). Duration of exposure for seed hardening is also counted important for better results. Improving germination by thermal treatment is proved to be important tool and may use for seeds which have poor quality. In our present study, attempt was made to achieve a better germination of chickpea with different temperatures.

MATERIAL AND METHODS

Seeds of the chickpea (*Cicer arietinum* L.) Kabli cultivar Noor-91 and desi cultivar Punjab-91 were used as the experimental material. Seeds of both cultivars were obtained from the Pulses Research Institute, Ayub Agriculture Research Institute (Faisalabad, Pakistan).

Thermal hardening treatments include dry heating 25 seeds of each cultivar incubated at 40, 50 and 60 °C for 24, 48 and 72 hrs each in an oven. Seeds were incubated in glass jars tightly covered with lids.

After treating seeds with different temperatures for different durations, these were sown in Petri plates between layer of filter paper which were moisturized with water and kept at 27 °C in an incubator. To record germination characteristics, seeds were observed daily according to the methods of the Association of Official Seed Analysis (1990). The time taken to complete 50% germination (T_{50}) was computed according to the formula of Coolbear et al. (1984) and modified by Farooq et al. (2004):

$$T_{50} = t_i + \left[\frac{N/2 - n_i}{n_j - n_i} \right]$$

Where N is the final number of germinated seeds and n_j and n_i are the cumulative number of seeds germinated by adjacent counts at times t_j and t_i , respectively, when $n_i < N/2 < n_j$.

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds that had germinated on day D and D is the number of days counted from the beginning of germination.

The germination index (GI) was determined as described by the Association of Official Seed Analysis (1983) using the following formula:

Germination Index

$$(GI) = \frac{\text{No. of germinated seeds}}{\text{Day of first count}} + \dots + \frac{\text{No. of germinated seeds}}{\text{Day of final count}}$$

The energy of germination (GE) was recorded on the 4th day after planting of seeds. This is the percentage of germinating seeds 4 d after planting relative to the total number of seeds tested (Ruan et al., 2002).

Secondary roots were counted daily to determine the rate of secondary root emergence. While on 10th day after sowing, the seedlings were tested for vigor after careful removal. Radicle and plumule lengths were recorded of 5 randomly selected seedlings per replicate and averaged. Seedling fresh weight was noted by weighing seedlings with digital balance and dry weight was taken after drying the seedlings at 70 °C for 7 days.

Graphical presentation of the data was made in Microsoft Excel. Standard error bars were computed to compare the real difference amongst the treatments.

RESULTS

Heating chickpea seeds improved germination and stand establishment of seedlings. In both chick pea cultivars, treated seeds were germinated earlier compared with untreated

(control) seeds. However, both cultivars responded different for different temperatures with different durations. In Kabli, seeds subjected to temperature of 50 °C for 48 or 72 h germinated earlier than seeds subjected to other treatments while desi type chickpea responded best at lower temperature i.e. 40 °C for 24 and 48 h. The highest T_{50} was noted in seeds treated with high temperature of 60 °C for all the durations (Fig 1). A similar trend was observed in case of mean germination time in both chickpea cultivars. Kabli chickpea responded best at 50 °C for 48 and 72 h while desi chickpea seeds at 40 °C for 24 and 48 h. Seeds treated with high temperature of 60 °C could not differ significantly from untreated seeds or control (Fig 1). In case of final germination percentage, seed treatments did not significantly affect; however, in temperature of 60 °C for all duration, significantly less germination percentage was observed as compared with control. The energy of germination was improved significantly by temperature treatments up to a certain limit. Both cultivars responded differently for energy of germination with different temperatures. Kabli chickpea seeds were observed with significantly more germination energy at temperature of 40 °C for 72 h and 50 °C for 24 h. In all other treatments of 40 °C or 50 °C, kabli seeds remained at par with control while temperature more than 50 °C significantly reduced the energy of germination. In desi chickpea seeds, germination energy was significantly high with treatments of 40 °C for 24 and 48 h. All treatments of 40 and 50 °C were at par with that of control while temperature of 60 °C significantly reduced the energy of germination (Fig 1). Similar trend was in case of germination index in both chickpea cultivars. In kabli chickpea, maximum germination index was recorded in seeds subjected to temperature of 40 °C for 72 h and 50 °C for 24 h, then followed by the remaining treatments, except control and 60 °C for all durations where germination index was

significantly lower than control. Germination Index of desi chickpea seeds was high in temperature of 40 °C for 24 and 48 h. All other temperature treatments remained at par with control except 60 °C which could not improve the germination index (Fig 1).

Radicle length was drastically improved by pre sowing heating treatments (Fig 2); however, minimum radicle length was noted in untreated seeds. Although different thermal treatments improved radicle length of chickpea, however, seeds of kabli heated at 40 °C were noted with longer radicle than all other. Whereas radicle length was decreased with increasing temperature and duration and at 60 °C radicle length of kabli was improved when seeds were heated for 24 hrs while in case of desi cultivar, radicle length was less than that of control. The plumule length of seeds undergoing the heating treatment protocols was higher at 40 and 50 °C while it was reduced when seeds were treated at 60 °C especially for a long time (Fig 2). However, among different treatments, heating seeds for 24 hrs at 40 and at 50 °C were found better than others.

Number of secondary roots were significantly improved with thermal hardening of chickpea seeds (Fig 3). Different hadening treatments were found effective in improvement of secondary roots, however, heating seeds at 40 and 50 °C for any time duration was most effective for improvement than control. Although heating at 60 °C for 24 hrs was also improved number of secondary roots but a longer duration viz. 48 and 50 hrs could found effective rather these were found harmful (Fig 3).

Fresh and dry weights of chickpea seedlings were improved with thermal hardening (Fig 4). Heating seeds at 40 and 50 °C for 24 hrs to 72 hrs were found effective in improving both fresh and dry weights of both chickpea cultivars and all these treatments were found at par. However, heating at 60 °C could not increase fresh or dry weight of any cultivar (Fig 4).

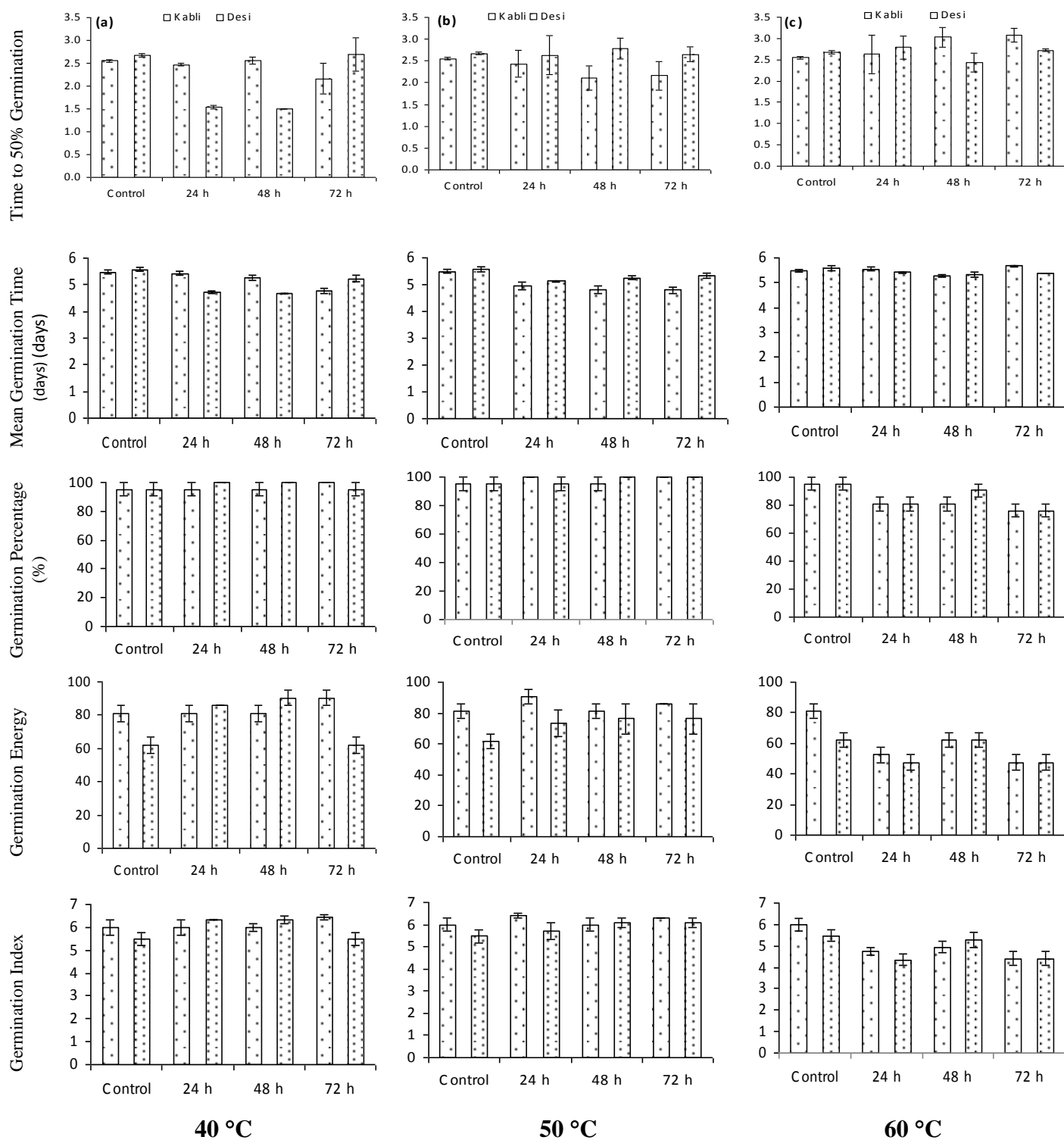


Fig. 1: Influence of dry heat treatments at (a) 40 (b) 50 and (c) 60 °C on the germination traits of chickpea cultivars

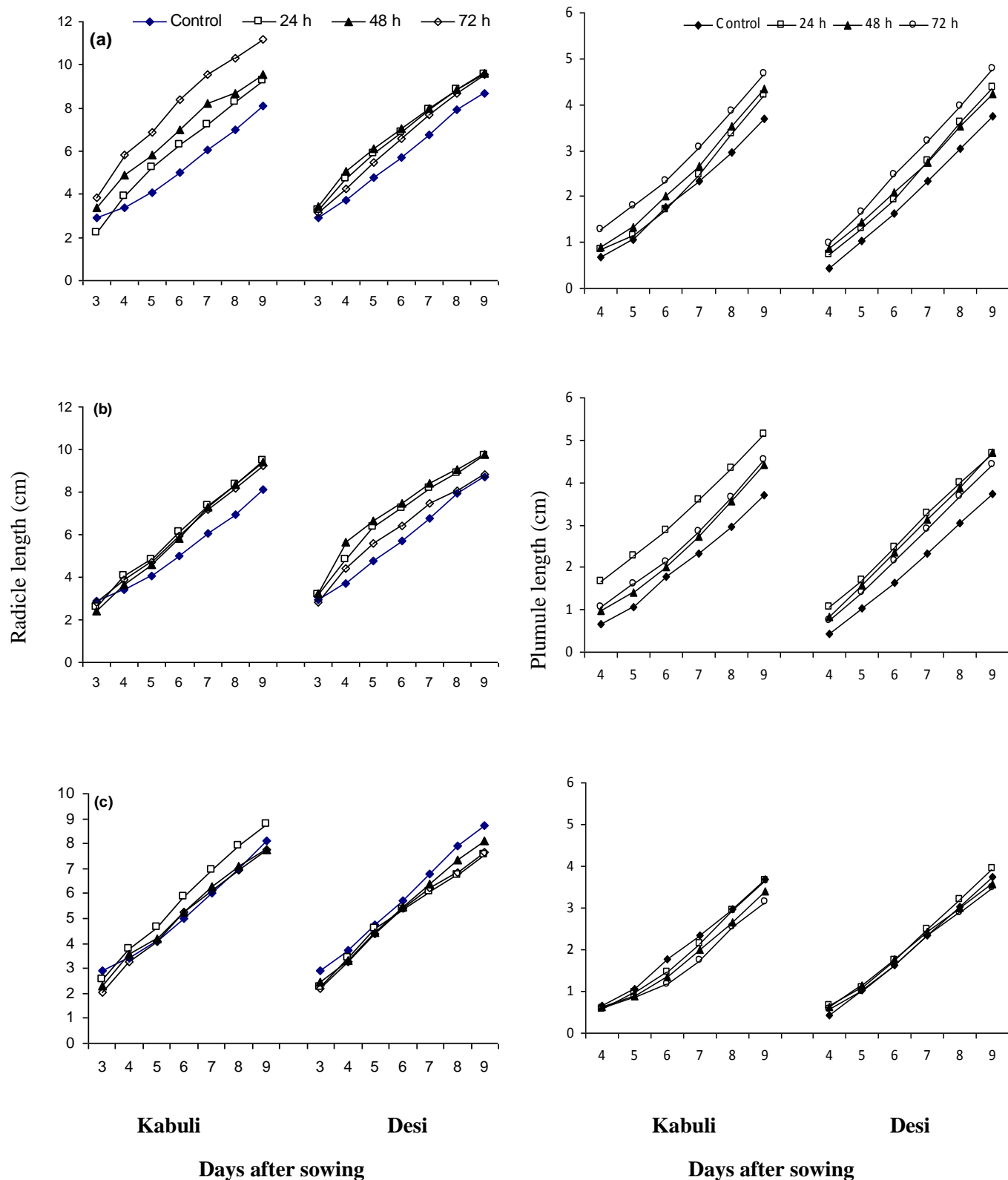


Fig 2. Influence of dry heat treatments at (a) 40 (b) 50 and (c) 60 °C on the radicle and plumule length of chickpea

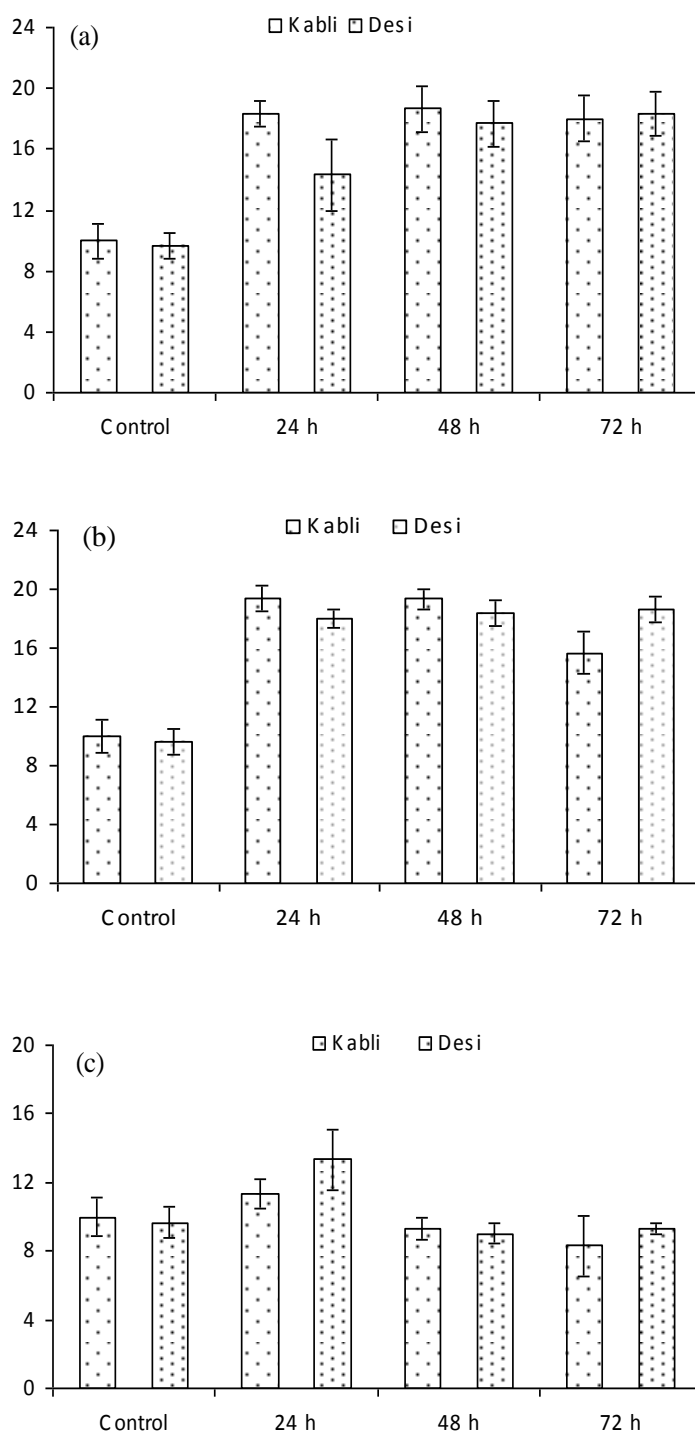


Fig 3. Influence of dry heat treatments at (a) 40 (b) 50 and (c) 60 °C on number of secondary roots of chickpea

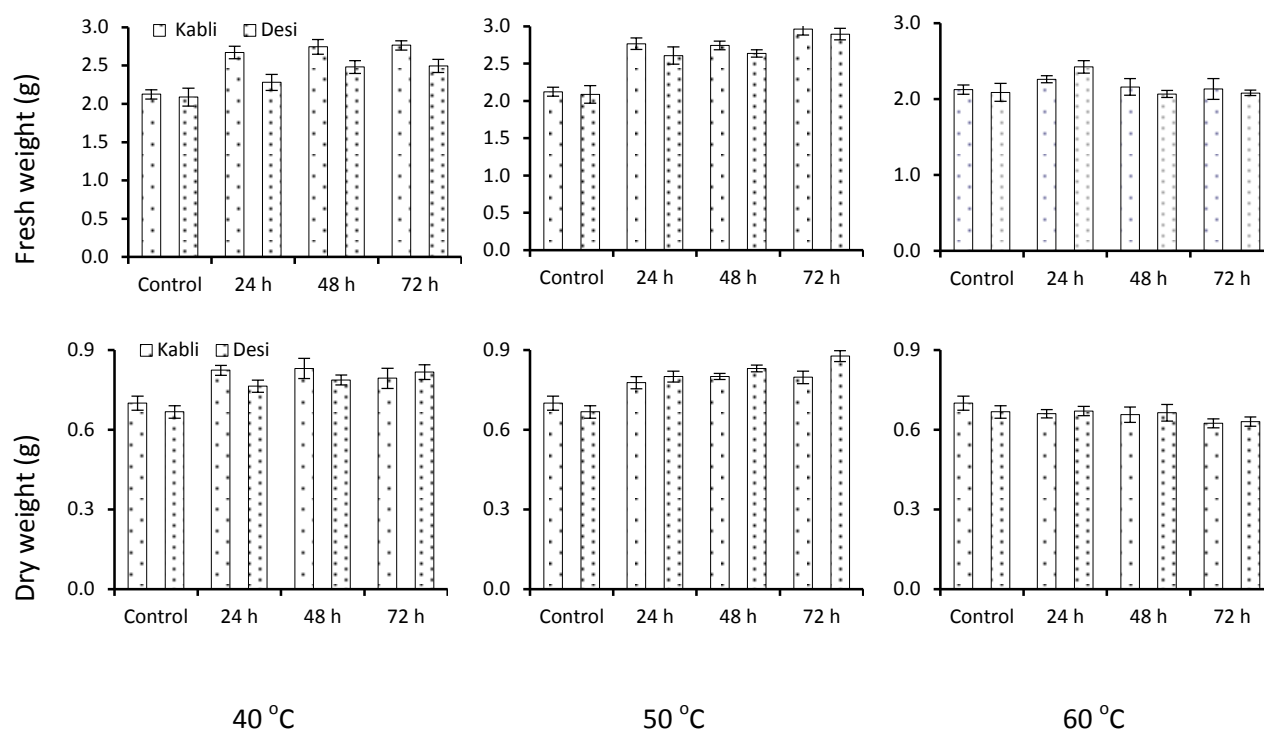


Fig 4. Influence of dry heat treatments at (a) 40 (b) 50 and (c) 60 °C on the fresh and dry weight of chickpea

DISCUSSION

Thermal hardening found to have a significant effect on the germination and seedling vigor of both chickpea cultivars (Fig 1, 2). However, the responses of both cultivars of chickpea to different dry heat treatments were not similar (Fig 1, 2). In both cultivars, earlier and synchronized germination was observed in treated seeds as compared to control seeds, as evidenced by a lower time to start germination, T_{50} , MGT and a higher GI, GE, FGP, lengths of radical and plumule, number of secondary roots as well as seedling fresh and dry weight (Fig 1, 2). Germination percentage and radicle length remained unaffected, but higher germination energy was recorded in treated seeds. It is well postulated that several seed treatments have to improve different germination traits of different crop species. Ruan et al. (2002) have also reported that seed treatments were not able to invigorate rice seeds, but resulted in a higher energy of germination and germination index compared with untreated seeds. Improvement in germination was observed due to some beneficial aspects primarily are due to pre-enlargement of the embryo (Austin et al., 1969), improvement of the germination rate (Gray and Steckle, 1977), and effects that are

attributed to alternating wetting and drying processes (Basra et al., 2003). Greater enhancement was noted in seeds subjected to dry heat treatment as compared to those seeds treated with chilling (Farooq et al., 2005). Farooq et al. (2004) also reported vigor enhancement in indica rice seeds subjected to dry heat treatment. However, these improvements was reported mainly in rice seeds while our study was on chickpea seeds. In our study is evident from the results that heat treatments significantly improved not only germination traits but also further improved number of secondary roots, radicle and plumule length and fresh and dry weights of both chickpea cultivars (Fig 1,2,3). Treating seeds with a higher temperature (60 °C) for long duration (72 hrs) was observed harmful indicating that chickpea seeds cannot tolerate a high temperature and increasing temperature from 60 °C will definitely damage the seeds. However, treating at high temperature for short duration improved different traits than control. Earlier Mott et al. (1982) reported that treating seeds with very high temperature 160 °C for seconds or a few minutes improved germination.

Improved fresh and dry weights are a result of earlier and healthy germination, which

contributed to further growth of chickpea cultivars. There was a little difference in chickpea cultivars in response to heat treatments, which is due to their genetic makeup. Genetic difference is found a reason for differential response to different treatments in some other species like rice (Lee et al., 2002).

In conclusion, heat treatment (upto 50 °C for not more than 72 hrs) are helpful in improving the germination of chickpea seeds.

REFERENCES

- Association of Official Seed Analysis, 1983. Seed Vigor Testing Handbook. Contribution No. 32 to the Handbook on Seed Testing. Association of Official Seed Analysis, Springfield, IL.
- Association of Official Seed Analysis, 1990. Rules for testing seeds. *J. Seed Technol.*, 12: 1–112.
- Austin RB, Longden PC and Hutchinson J, 1969. Some effects of “hardening” carrot seed. *Ann Bot* 33, 883–895.
- Basra SMA, Farooq M and Khaliq A, 2003. Comparative study of pre-sowing seed enhancement treatments in fine rice (*Oryza sativa* L.). *Pak. J. Life Soc. Sci.* 1: 5-9.
- Basra SMA, Farooq M and Tabassum R, 2004. Physiological and biochemical aspects of seed vigor enhancement treatments in fine rice (*Oryza sativa* L.). *Seed Sci. Technol.*, 33: 21.
- Cavanagh T, 1987. Germination of hard seeded species (Order Fabales). *In: Langkamp, E.P. (Ed.). Germination of Australian Plant Seed*, Inkata Press, Melbourne, pp. 58–70.
- Coolbear P, Francis A and Grierson D, 1984. The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds. *J. Exp. Bot.*, 35: 1609-1617.
- Ellis RA and Roberts EH, 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci Technol* 9: 373–409.
- Farooq M, Basra SMA, Hafeez K and Warriach EA, 2004. Influence of high and low temperature treatments on the seed germination and seedling vigor of coarse and fine rice. *Int. Rice Res. Notes*, 29: 69–71.
- Farooq M, Basra SMA, Ahmad N and Hafeez K, 2005. Thermal Hardening: A New Seed Vigor Enhancement Tool in Rice. *J. Integ. Plant Biol.*, 47: 187–193.
- Gray D and Steckel JRA, 1977. Effects of pre-sowing treatments on the germination and establishment of parsnips. *J Hort Sci* 52, 525–534.
- Khan MA, Akhtar N, Ullah I and Jaffery S, 1995. Nutritional evaluation of desi and kabli chickpeas and their products, commonly consumed in Pakistan. *Int. J. Food Sci. Nutr.*, 46: 215–223.
- Lee SS and Kim JH, 2000. Total sugars, α -amylase activity, and germination after priming of normal and aged rice seeds. *Kor. J. Crop Sci.*, 45: 108–111.
- Lee SY, Lee JH and Kwon TO, 2002. Varietal differences in seed germination and seedling vigor of Korean rice varieties following dry heat treatments. *Seed Sci Technol* 30, 311–321.
- Mott, JJ, Cook SJ and Williams RJ, 1982. Influence of short duration high temperature seed treatment on the germination of some tropical and temperate legumes. *Tropical Grasslands*, 16: 50-55.
- Muehlbauer FJ and KB Singh, 1987. Genetics of chickpea. *In: Saxena, M.C. and K. B. Singh (Eds.). The Chickpea*. Wallingford, OX: CAB International. pp. 99–125.
- Pe´rez F and Pita JM, 1997. Ecofisiologı´a de la germinacio´n de las jaras (*Cistus* spp.). Lecciones hipertextuales de botanıca (revista@quercus.es).
- Ruan S, Xue Q and Tylkowska K, 2002. The influence of priming on germination of rice (*Oryza sativa* L.) seeds and seedling emergence and performance in flooded soils. *Seed Sci Technol* 30, 61–67.
- Thanos CA and Georghiou K, 1988. Ecophysiology of fire stimulated seed germination in *Cistus incanus* ssp. *creticus* (L.) and *C. salvifolius* (L.). *Plant Cell Env.*, 11: 841–849.
- Tran VN and Cavanagh AK, 1980. Taxonomic implication of fracture load and deformation histograms and the effects of treatment on the impermeable seed coats of *Acacia* species. *Aust. J. Bot.*, 28: 39–51.
- Whelan RJ, 1995. *The Ecology of Fire*. Cambridge University Press, Cambridge.