Impact of high temperature on the reproductive stage of chickpea

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Abstract

Chickpea (*Cicer arietinum* L.) is a high-value pulse crop. It is widely cultivated under a range of climatic conditions. Sowing time may vary in different locations depending on the temperatures experienced at different stages of crop development. Temperature is therefore the most important growth parameter that governs yield and high temperatures during the reproductive stage in chickpea is a major cause of yield loss. Global warming is predicted to increase temperatures by up to 5?C by the end of this century, with associated changes in mean maximum temperature. The objective of this research is to study the effect of high temperature during pre- and post-anthesis stages of flower development on pollen viability, pollen germination, pollen tube growth and pod set. The plants were evaluated under two contrasting environments viz., heat stress and non-heat stress in controlled environments. Heat stress (35/20 and 40/25?C) was applied at the flowering stage to plants grown in the growth chamber to study pollen and pistil behaviour including flower abortion and pod set, compared with the control (28/16?C). The high temperatures reduced pod set by reducing pollen viability. The pollen of tolerant line (ICCV92944) was fully viable at 35/20?C and partially viable at 40/25?C, while the pollen of sensitive line (ICC5912) was fully sterile at 35/20?C. These results suggest that selection for pollen viability could improve the heat tolerance of chickpea.

Keywords

Anther, heat stress, pollen viability, pollination, pre-anthesis, post-anthesis

Introduction

Chickpea is an important grain legume, grown either under rainfed conditions largely on stored soil moisture (Summerfield et al. 1990) or with irrigation (Saxena et al. 1996). In both conditions, high temperatures at the reproductive stage adversely affect the yield. Temperature is a key environmental factor for crop growth, development and adaptation of chickpea (Thomson et al. 1997). The effect of temperature on grain yield is expected to increase due to global warming. A minimum decrease of 53 kg/ha of chickpea yield was observed in India per 1?C increase in seasonal temperature (Kalra et al. 2008). Whilst chickpea is considered to be a cool season legume, temperatures >30?C are common during the reproductive stage in the semi-arid tropics of India, in the summer dominant rainfall region of northern New South Wales and also in southern Australia. An understanding of heat tolerance is important for the adaptation of chickpea to these regions. Therefore it is essential that the most sensitive stages of the reproductive phase to high temperature are identified.

Short (10 days) periods of high temperatures (≥35?C) during early flowering and pod development of chickpea are known to cause significant reduction in pod, seed set and yield (Wang et al. 2006). The yield reduction was the result of high temperature effects on pre-anthesis, pollination and post-anthesis development. The anthers have been reported to be influenced by high temperature, thus adversely affecting pollination and eventual groundnut grain yield (Prasad *et al*. 1999). This research was conducted to identify the effect of high temperature during pre- and post-anthesis stages of flower development on pollen viability, pollen germination, pollen tube growth and pod set in chickpea.

Materials and methods

Two controlled environment experiments were conducted with two chickpea genotypes (ICCV92944 and ICC5912) at the International Crops Research Institute for the Semi-arid Tropics, (17.53?N;?78.27?E; ?545 m) India. Plants of the two genotypes were grown in a controlled environment with five replications. Seeds of each genotype were sown in pots (2.4 L volume) containing a mixture of black vertisol soil, sand and vermi-compost (4:2:1 by volume). The phenology of the two genotypes was different. To overcome this problem, staggered sowing was conducted in the glasshouse. The plants were grown at 28/16?C in a greenhouse and transferred to a growth room at the first appearance of flowers to expose them to high temperature. The plants used as a non-stressed control continued to grow in the glasshouse at 28/16?C. The temperature in the growth room was increased daily by 1?C, e.g. 29 to 40?C during day and 16 to 25?C during night (Table 1). Therefore, the plants were exposed to a gradual increase in temperature.

Experiment-1

The effects of a one day exposure to temperature from 31/16?C to 35/20?C during pre-anthesis were studied to determine the critical temperature. One day before anthesis (pre-anthesis), five flower buds were collected between 08:00 and 08:15 h from 31/16?C to 35/20?C to examine pollen viability. The heat tolerant genotype was examined at 40/25?C (extreme temperature) for pollen viability. Anthers stained with Alexander stain and examined under electron microscope are shown in Fig. 1 and Fig. 2. The fertile pollen grains inside the anthers were red in colour whilst the sterile pollen grains were green. Flowers at 35/20?C were tagged to observe the pod set. ANOVA was performed for flower data using Genstat 12th Ed. VSN International Ltd.

Experiment-2

Following experiment-1, hand pollination was used in the 35/20?C treatment to achieve pollination (post-anthesis). Five flowers were collected from 15mins and 30mins after pollination to observe the pollen germination on the stigma and pollen tube growth down the style. The flowers were fixed for 24 h in 80% alcohol. The pistils (styles and ovary) were removed from the flowers, cleared with 6N NaOH for 48 h and thoroughly rinsed with water. The pistils were stained with aniline blue and observed under a fluorescence microscope.

Table 1. Details of temperature regime, pre-anthesis, and post-anthesis of chickpea flower collection under controlled environments.

Days	Temp regime (day and night - ?C)	Flower buds collected one day before anthesis to check critical temperature (pollen viability)	Post-anthesis (Pod set) observation	Hand pollination to study pollen germination and pollen tube growth
Day 1	29/16	-	-	-
Day 2	30/16	-	-	-
Day 3	31/16	?	-	-
Day 4	32/17	?	-	-
Day 5	33/18	?	-	-

Day 6	34/19	?	-	-
Day 7	35/20	?	?	?
Day 8	36/21	-	-	-
Day 9	37/22	-	-	-
Day 10	38/23	-	-	-
Day 11	39/24	-	-	-
Day 12	40/25	?	-	-

Note: The symbol ? indicates the day of sample collection

Results and discussion

The one day exposure of flower buds to high temperature influenced pollen viability inside the anthers. In ICC5912, the pollen grains inside the anthers were fertile up to 34/19?C (Fig. 1a). At 35/20?C, all pollen grains inside the anther became sterile (Fig. 1b). This difference was similar in all 10 anthers positioned inside a flower. In addition, there was no pod set in ICC5912 at 35/20?C. In ICCV92944, the high temperature of 35/20?C did not influence pollen fertility and the pollen grains were fertile (Fig. 2b). At 40/25?C, the pollen grains inside the anther were partially sterile in ICCV92944 (Fig. 2c). Therefore, the critical temperature for pod set in ICC5912 was 35/20?C. The pollen viability in chickpea was affected by high temperature of ≥35?C, which concurs with Prasad *et al*. (1999) working with groundnut. Similarly, the pod set and yield in chickpea was reduced and associated with poor pollen viability due to exposure to high temperatures at microsporogenesis (Warrag and Hall 1984). Pollen germination and pollen tube growth was found in ICCV92944 at 35/20?C (Fig. 3a, 3b). Though the pollen tube had callus formation, the tube reached the mouth of the ovary at 30mins (Fig. 3c). However there was no pollen germination on the stigma of ICC5912 at 35/20?C. At 35/20?C, ICC5912 had reduced pod set (P<0.001) compared with ICCV92944 and the control (28/16?C) (Table 2). These results confirm the heat tolerance of ICCV92944 and heat sensitivity of ICC5912. The yield reduction was linked with the pollen trait. Consequently, there is potential for developing the screening techniques for heat tolerance in the field and in the controlled environments for chickpea breeding programs using the differences in pollen viability. There is also a possibility of using a pollen selection method in breeding for heat tolerance. Further research is needed to investigate more germplasm and to confirm these results.

(c)

(a)

(b)

Sterile

Sterile

Fertile

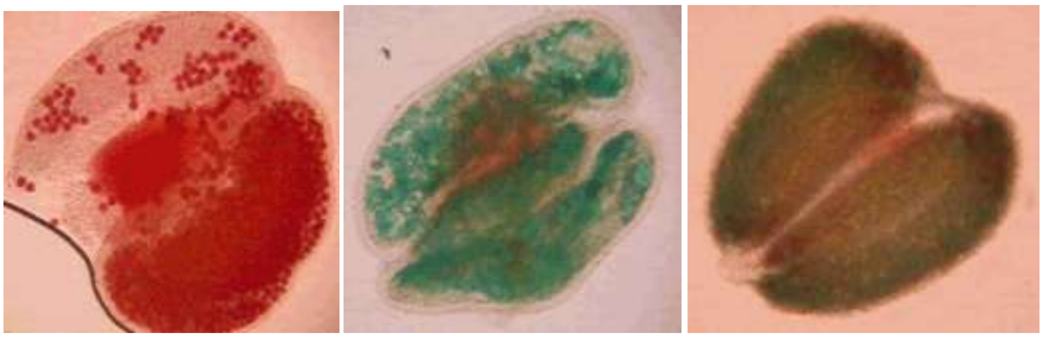

Fig.1 ICC5912 (a) Anther-34/19?C (pollen grains are fertile) (b) Anther-35/20?C (pollen grains are sterile)

(c) Anther-40/25?C (pollen grains are sterile) (Fertile pollen grains are red; Sterile pollen grains are green)

Sterile

(c)

(b)

(a)

Fertile

Fertile

Fertile

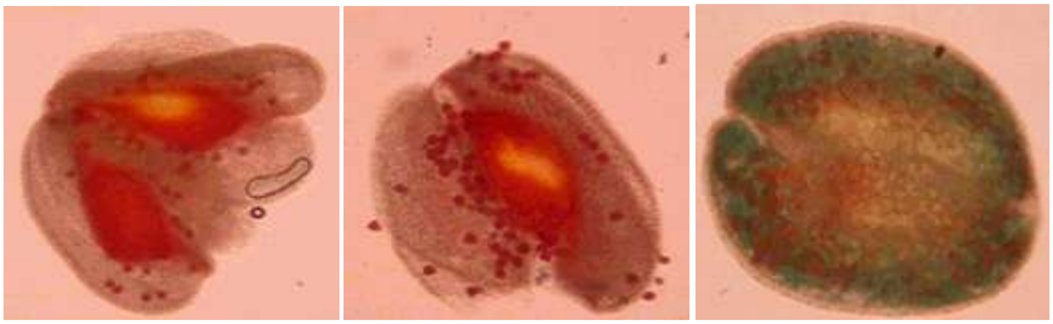

Fig. 2 ICCV92944 (a) Anther-34/19?C (pollen grains are fertile) (b) Anther-35/20?C (pollen grains are fertile)

(c) Anther-40/25?C (pollen grains are fertile and sterile) (Fertile pollen grains are red; Sterile pollen grains are green)

Trichome

Pollen tube growth near ovary

(c)

Pollen germination

Pollen tube growth with callus

(b)

(a)

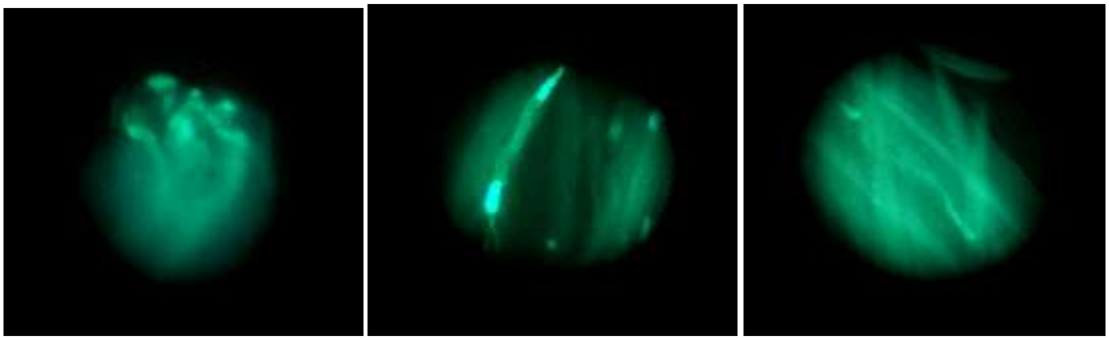

Fig. 3. ICCV92944-(a) Pollen germination on stigma 15min after pollination (b) Pollen tube growth on style with callus 15mins after pollination (c) Pollen tube growth near the mouth of the ovary 30mins after pollination (Trichome is present at the mouth of the ovary).

Conclusions

Hot days (≥35?C) and warm night (≥20?C) temperature can potentially reduce the pollen viability and subsequent pod set in heat sensitive chickpea genotype. Reduced pod set was a consequence of poor pollen viability. The critical temperature for pollen viability was ≥35?C in the heat sensitive chickpea genotype. Therefore, pollen viability at high temperatures is a possible indirect selection criterion to improve the heat stress tolerance in chickpea.

Table 2. Comparison of high (35/20?C) and optimum (28/16?C) temperature on flowers under controlled environments (Significant difference at *****P*<0.01; ******P***<0.001 respectively and NS-Not significant; Means followed by different letters within the same column are significantly different at *P*=0.05)**

Genotypes	Temp regime (day/night) – (?C)	Total no of flowers	No of dry flowers	% of dry flowers	Total no of pods	% of dry pods	% of pod set (retain pods)
ICCV92944	28/16	14	1	7a	13	7	99a
ICCV92944	35/20	9	2	10a	7	1	93b
ICC5912	28/16	12	1	10a	11	3	87c
ICC5912	35/20	6	6	100b	0	0	0d
Temperature effect							
28/16		13	1a	9a	10a	2	93a

35/20	7	4b	60b	6b	4	46b
Genotype effect						
ICCV92944	12	1a	13a	12	4	96a
ICC5912	9	4b	55b	4	1	44b
Temperature LSD (*P*=0.05)	NS	** (1.4)	*** (7.9)	** (4.0)	NS	*** (3.7)
Genotype LSD (*P*=0.05)	NS	** (1.4)	*** (7.9)	NS	NS	*** (3.7)
Genotype x Temperature LSD (*P*=0.05)	NS	NS	*** (11.2)	NS	NS	*** (5.3)

Note: values in parenthesis indicate the LSD at *P*=0.05

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