High-temperature Stress and the Fate of Pollen Germination and Yield in Lentil (Lens culinaris Medikus)

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ABSTRACT

Background: High temperatures adversely affect the growth and development of plants at vegetative, reproductive and maturity stages of their life cycle. The maximum air temperature of higher than 25°C frequently encountered in lentil at the reproductive stage mainly in West Bengal rice-lentil cropping pattern. Such high temperatures in rabi season impairs the crop growth and productivity. Added to this, future climate predictions are indicating 27-28°C during this period.

Methods: The field-laboratory experiment was conducted during 2014, with 20 genotypes of lentil. The field experiment was laid in split plot design with 3 different windows of sowing, while the laboratory investigation was carried out in completely randomized design with pollen collected from the field experiment.

Result: The present investigation reveals that high air temperature >25°C resulted in the failure of pollen germination, increase in abnormal pollen tube and aborted pollen. A drastic effect in seed filling and pod development in lentil was observed. Out of 20 lentil genotypes studied, ILL-10893 and L-13-113 emerged as tolerant genotypes while, BM-6 and L-4076 as susceptible with special emphasis on physiological characters.

Key words: High-temperature stress, Lentil, Pollen germination, Rabi, Reproductive growth, Yield.

INTRODUCTION

Lentil is an annual food legume highly valued for its grain, rich in protein and micronutrients for human consumption. India is contributing more than three fourth of the total world’s lentil production (Alexander, 2015). Lentil is sown as a cool-season crop and is highly susceptible to higher temperatures when sowings are delayed. It needs low temperatures during vegetative growth, while at maturity requires warm temperatures. The best temperature for its optimum growth is 18-30°C (Roy et al., 2012). The main constraints of production in pulse crops, including lentil, are their sensitivity to the effects of heat stress at the reproductive stages of development when plants are in full bloom (Erskine, 1996).

Heat stress causes various physiological changes in lentil such as leaf and stem scorching, leaf abscession and senescence, shoot and root growth inhibition, reduction in the number of flowers, inhibited pollen tube growth, pollen infertility and fruit damage, leading to catastrophic losses in crop yields. Reproductive development (flowering and seed filling) is most susceptible to high-temperature stress and rise in temperature during flowering by a few degrees can lead to complete crop loss (Asseng et al., 2011). Temperatures above 32/20°C (max./min.) during flowering and pod filling in lentil can drastically reduce seed yield and quality (Delahunty et al., 2015).

In West Bengal conditions, lentil cultivation is delayed in rice-lentil cropping sequence due to the late onset of monsoon, which leads to delay in sowing and harvesting of kharif rice; as a result, a period of substantially high temperature often coincides with the reproductive and pod filling phase of the crop. Even a few days of high temperature (30-32°C) may cause flower and pod abortion, resulting in yield losses by reducing seed set, seed weight and accelerating senescence in this crop (Gaur et al., 2015). In this experiment, an attempt was made to access the fate of pollen under heat stress, screen and select lentil genotypes suitable for delayed sowing conditions by prioritizing pollen germination, pot set and yielding capacity.

MATERIALS AND METHODS

The field experiment was conducted in District Seed Farm, AB Block, Kalyani, Nadia, West Bengal, India. The latitude is 22° 58’ N and longitude is 88° 32’ E with an altitude of 9.75m above mean sea level. The soil texture of the experimental plot was sandy loam with pH 6.9-7.0. Twenty genotypes of lentil were sown on three different dates viz., 15th November (1st sowing), 6th December (2nd sowing) and
27\textsuperscript{th} December (3\textsuperscript{rd} sowing) of 2014 as usually recommended sowing, moderately delayed and delayed sowings respectively.

The field experiment was laid in split-plot design, replicated thrice considering sowing dates in the main plot and genotypes in sub-plot. Appropriate plant protection measures, along with other standard cultural practices, were followed to raise a healthy crop. The crop was raised with residual moisture present in the soil, without application of any irrigation. In-vitro pollen germination studies were carried out in CRD (Completely randomized design).

**Weather data**

Standard week meteorological data during the crop growth period of 2014-15 was presented in Fig 1 and Fig 2. Highlighted boxes represent the key reproductive phase in respective sowings, 1\textsuperscript{st} sowing- Green, 2\textsuperscript{nd} sowing-Blue and 3\textsuperscript{rd} sowing-Red. The minimum, mean air temperature (Fig 1) and mean relative humidity and rainfall (Fig 2) were recorded between 12 November 2014 to 18 March 2015. The mean min./max. temperatures of all three sowings particularly for key reproductive phase were 26.66/11.63, 28.70/11.52 and 29.30/12.52\textdegree C (max./min.) respectively.

Total rainfall received during the crop growth period is 24.2 mm in all the sowings, which was received in 5 rainy days. The normal sowing was benefited with the first rainy day with 2.5 mm rainfall just before initiation of the reproductive stage whereas the rainfall received in other 4 rainy days have benefitted the crop to a little extent, as it was after pod formation stage in all the sowings, as clearly shown in Fig 2.

**Measurement of soil water potential**

Soil water content was measured gravimetrically at 0-15 and 15-30 cm depth at 35 DAS, 50 DAS and 65 DAS as per Pal, (2013) in all three sowings. The soil water potential was measured by using a pressure plate apparatus following Richards (1948). The data on soil moisture potential presented in Fig 3.

**Studies on pollen germination**

Flowers were collected from 10 plants per genotype at the time of anther dehiscence, between 06:00 am and 07:00 am, immediately placed in Petri dishes lined with moistened filter paper to avoid pollen desiccation. Pollen collected from 10 randomly selected flowers with the help of needle was dusted on a slide and mixed using a nylon hairbrush, then transferred on to the growth medium prepared as per Niles and Quesenberry, (1992).

Slides with media and pollen were placed in Petri dishes lined with moist filter paper, thus serving as germination chambers. The growth media on slides were placed in the incubator 15 minutes prior to pollen inoculation. The Petri dishes with slides containing pollen were maintained at 16\textdegree C (ambient), 24\textdegree C and 32\textdegree C in the incubator under dark condition.

A pollen grain was considered as germinated when the length of the pollen tube was equal to or longer than the diameter of the pollen. Counts were made at random in three fields for each slide under low power (10X) microscope. Pollen germination was recorded at 30 minutes interval up to 90 minutes during which the pollen reached the maximum percentage germination. There were five replicates for each genotype under each temperature treatment. The final percentage of germination was calculated as:

\[ \text{Germination} \% = \frac{\text{Number of germinated pollen per field}}{\text{Total number of pollen per field}} \times 100 \]

**Studies on reproductive efficiency**

Screening of genotypes for heat escaping or avoidance based on reproductive efficiency. Ten plants from each replication of each genotype were tagged for the study. Total numbers of flowers were counted every day. At the time of harvest, pod number, flower to pod ratio, seed size and yield was recorded.

**Statistical analysis**

The mean values for all the characters were analyzed following a split-plot design with three replications for field experiment and completely randomized design for in-vitro testing with five replications by INDOSTAT version 7.1 software. Graphs were illustrated using software GraphPad Prism version 7.0.

**RESULTS AND DISCUSSION**

Effect of high-temperature stress on in-vitro pollen germination

The in-vitro pollen germination percentage of the lentil genotypes was recorded at 30, 60 and 90 minutes after incubation under varying temperatures (viz. 16, 24 and 32\textdegree C). Analysis of variance indicated highly significant variation among the temperature effects, genotypes as well as temperature x genotype interactions. The optimum pollen germination was recorded at 16\textdegree C and the high-temperature stress adversely affected the pollen germination in all genotypes. A steady slope decline in pollen germination was

![Fig 1: Standard week maximum and minimum temperatures during crop growth period 2014-15 (key reproductive phase of lentil, Green box-1st sowing, Blue-2nd sowing and Red-3rd Sowing).](image-url)
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observed with the increase in temperature; only a few genotypes were able to cross 20% pollen germination. The finding corroborated well with the early report of Barghi *et al.* (2013). The observed decrease in pollen germination at high temperature might be attributed to under-utilization of sucrose by pollen grains (Aloni *et al.*, 2001) or unavailability of starch and sugar in pollen grains at high temperature (Pressman *et al.*, 2002). The transformed data is presented in Table 1. The genotypes KLS-218, HULL-57, L-4076 and BM-7 showed 79.61, 83.06, 84.25 and 85.02% reduction in in-vitro pollen germination percentage, respectively, under the most extreme temperature (32°C) as compared to 16°C (Table 1). Thus, these genotypes were found to be the most sensitive to high-temperature stress in respect of pollen fertility and viability. It might be significant to note that these four genotypes registered more than 90% reduction in the number of pods per plant on an average under the 3rd sowing date (Fig 4b), the genotypes were exposed to (>30°C) the most extreme level of high-temperature stress in the reproductive stage.

On the contrary, the genotype ILL-10893 with 10.51 and 29.48% reduction in pollen germination at 24 and 32°C respectively (Fig 5), established itself to be the least affected by high temperature and they registered comparatively lower reduction (63.83%) in pods per plant. The genotype L-13-113 registering 62.80% reduction in pollen germination percentage at extreme temperature, revealed only 39.50% reduction in the number of pods per plant under 3rd sowing. It might be concluded that although L-13-113 had moderate sensitivity to temperature extreme in respect of pollen viability, it ultimately managed to escape from the extreme conditions of terminal heat and drought stress due to its extra earliness in flowering.

**Effect of terminal heat and drought stress on flower production and pod set**

The decline in the availability of soil moisture (Fig 3) along with the increasing temperatures leads to heat-induced drought stress. Data on the number of flowers per plant and pod setting percentage indicated highly significant variation among the sowing dates, genotypes as well as their interaction effects. Perusal of the data revealed that both the flower number and pod setting percentage reduced under late sowing dates as compared to normal sowing with
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Fig 4a: % Change over 1st sowing in moderately delayed sowing, 4b. % Change over 1st sowing in delayed sowing. on x-axis 1-20 indicates sequence of genotypes mentioned in table 1 and 2. (X1-Flower/plant, X2-Pod/plant, X3-Pod set, X4-100 seed weight (g) and X5-Seed yield/plant).

Fig 5: Pollen germination variability of two tolerant (ILL-10893 and L-13-113) and two susceptible (BM-6 and L-4076) lentil genotypes at 16, 24 and 32°C.
In the present experiment, the twenty genotypes of lentil registered a gradual decline in respect of five important yield attributes viz., the number of flowers per plant, pods per plant, pod set, test weight (100-seed weight) and seed yield per plant (Table 2) and % change over 1st sowing was depicted in Fig 4a and 4b for second and third sowings, respectively. All these five characters were drastically affected under the 3rd sowing date with the number of pods per plant and seed weight per plant being the most severely affected. Significant reduction in seed yield under water deficit was also reported by Shrestha et al. (2006); Azizi et al. (2006); Azizi et al. (2006) and Barghi et al. (2006) who also noted that the pod setting percentage in L-13-113 improved all the genotypes under late sowing dates. It was significant to observe that the number of flowers per plant, pods per plant and test weight (100-seed weight) per plant being the most severely affected under the 3rd sowing date also registered the severe reduction in seed yield in the present study, an observation that corroborated with the reports of Barghi et al. (2013).

Perusal of interaction effects between sowing dates and genotypes indicated a few critical points. Sowing of genotypes on 26th December resulted in a most severe reduction in seed yield and its component characters in the lentil genotypes under study. The delayed sowing induced earliness in flowering in all the genotypes. This might indicate a general strategy of the genotypes for drought escape under the moisture-depleting condition in rainfed farming. The observation was well consistent with that of Silim et al. (1993), who suggested that drought escape was the key response to drought stress. In the present experiment, the three genotypes, KLS-218, HULL-57 and L-4076 with a most drastic reduction in the number of mature pods per plant under 3rd sowing date also registered the severe reduction in seed yield in each plant of 97.15, 98.51 and 98.12%, respectively (Fig 4b), making these genotypes most sensitive to terminal heat and drought stress. Thus, it was evident that the reduction in pod number was the key determiner for yield reduction in these genotypes of lentil under terminal heat and drought stress. This finding confirmed the reports of Shrestha et al. (2006) and Barghi et al. (2013) who also observed...
found that reduction in the number of filled pods might be led to a significant reduction in seed yield in lentil under drought and heat stress imposed during the reproductive stage. It was significant to note that the genotype L-13-113 showed extra earliness in flowering under all the three sowing dates and it ultimately registered the highest tolerance to heat and drought stress in the reproductive stage. The duration of L-13-113 and ILL-10893 is 116 and 120 days in regular date of sowing, which reduced drastically due to heat stress. The earliness in flowering helped the genotype in escaping from drought and high-temperature stress at the end of the season and registering the highest adaptability among the genotypes for late sowing condition. The genotype L-13-113 showed 39.50 and 60.23% reduction in the number of pods per plant and seed yield per plant, respectively, under the latest sowing date (third sowing) as compared to the 1st sowing (Fig 4b). The findings of the experiment were altogether corroborated with the observations of Roy et al. (2012); Hojjat, (2013) and Cardenas-Travieso et al. (2014).

CONCLUSION

The study with 20 genotypes and three different date of sowing was observed that due to delay in sowings, a period of substantially high temperature often coincides with the flowering and grain filling phases of the lentil which causes flower and pod abortion, ultimately resulted in yield loss. Pollen germination at different temperature varies significantly, at increased temperatures pollen tube gets spiralled, coiled and further increase in temperature leads to bursting of pollen. It was found that genotypes of lentil ILL-10893 and L-13-113 escaped heat stress due to earliness in the reproductive phase.

ACKNOWLEDGEMENT

Authors are thankful to ICARDA for providing the necessary facilities for the field experiment. Authors are also grateful to Prof. V. Radha Krishna Murthy (ANGRAU) and Dr. Suman Roy (CRUJAF) for constructive suggestions in preparing the manuscript.

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