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# EFFECT OF TEMPERATURE STRESS ON *BRASSICA RAPA* GENOTYPES DURING GERMINATION AND REPRODUCTIVE GROWTH

**K.M.K. Huda[1*], M.S.A. Banu[2], M.H. Rashid[1], S. Parveen[1] and M.A.M. Hussen[2]**

[1]Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University (SAU), Dhaka; [2]Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka. Bangladesh

## Abstract

The effect of temperature stress on the reproductive development and yield of five *Brassica rapa* was studied in a controlled growth chamber. The seeds of five *Brassica* genotypes namely BARI Sarisha-6, Tori 7, Kallayania, BARI Sarisha-9 and BINA Sarisha-4 were grown under day/night temperatures at 22/17$^0$C until early flowering or early siliqua development stage. The high temperature stress i.e., 28 /17$^0$C or 35/17$^0$C, both at early flowering stage and early siliqua formation stage were imposed for 7d and then allowed to recover at 22/17$^0$C for normal growth. Shoot dry matter accumulation varied with temperature and genotype. The highest shoot dry matter was recorded by Kallayania followed by BARI Sarisha-9. The optimum day temperature for seed yield of the main shoot showed closer to 28$^0$C. The largest seeds were produced in the 22/17$^0$C, the size, however, remained similar with 28/17$^0$C temperature stress. During this temperature regime, the highest seed yield was observed with BARI Sarisha-6 while BARI Sarisha-9 produced the lowest seed yield. Severe temperature stress (35/17$^0$C) reduced seeds per siliqua and 1000 seed weight in all genotypes. Maximum pollen viability and germination were recorded at 20$^0$C by Kallayania followed by Tori 7 at 25$^0$C. However, the temperature above 30$^0$C reduced pollen viability and germination for all the genotypes. In another set of treatments, the high temperatures exposure (28/23$^0$C) for 5d reduced oil content and increased protein concentrations. Plant exposed to 25/20$^0$C temperature stress showed significant changes in oleic, linoleic, and linolenic acids. The levels of saturated fatty acids were similar across the five genotypes in the control treatment, but palmitic acid and stearic acids increased with the temperature.

**Keywords:** *Brassica*, Oil, Protein, Temperature stress, Yield

## Introduction

The global temperature is predicted to rise up to 5.8$^0$C as a result of increasing greenhouse gases in atmosphere, which can have harmful effect on crop yield in northern temperate areas where plants are adapted to grow in lower temperatures. Oilseed rape lines can greatly vary in their adaptation abilities in high temperature. This can in worst cases lead to reductions in yield and oil content during flowering (Aksouh *et al.,* 2006). Optimum temperature for yield formation can be lower than the optimum temperature for

* Corresponding Author: mashnuk@gmail.com

leaf appearance rate, vegetative growth, or reproductive progression yield (Peñuelas and Filella, 2001). High temperature often accelerates phenological development which can result in smaller plants, shorter reproductive phase, and lower yield. In addition to acceleration in life cycle and interrupted carbohydrate accumulation, reproductive stage including micro sporogenesis and mega sporogenesis is highly sensitive to high temperature and its disturbance can decline seed yield. When pollen or egg cell development is delayed or interrupted, fertilization fails and unfertilized ovules can become aborted (Seavey *et al.,* 2000). Short exposure (1-2 weeks) to high temperature ($35^0$C) for 4 h/day impaired pollen viability and seed set (Young *et al.,* 2004). Four-hour exposure daily for one week to $30^0$C depleted the pollen development and germination in turnip rape. Moreover, pollen development was slowed and germination ability of pollen from heat treated plants was also impaired. Short heat treatment of oilseed rape during seed filling influenced oil quality by decreasing the amount of saturated fatty acids and decreasing mono-unsaturated oleic acid content (Aksouh *et al.,* 2001). The number of siliquae in oilseed rape, as well as seed weight, was also affected by heat treatment and varied highly between different genotypes.

Research institutes in Bangladesh have developed improved verities of *Brassica* including BARI Sarisha-6, BARI Sarisha-9, BARI Sarisha-14, BARI Sarisha-15, Kallayania and BINA Sarisha-4 and improved Tori. In Bangladesh, mostly research are focused on different management practices for improving yield but less on stresses such as climate change, soil salinity, drought, flooding, metal toxicity, pollution, and extreme temperatures. The genotypes BARI Sarisha-6, BARI Sarisha-9, Kallayania and BINA Sarisha-4 and Tori are moderately waterlog tolerant but the effect of temperature on these genotypes was not yet investigated. Therefore the objectives of this study were to determine the response of five genotypes of *Brassica* to high temperature stress and to assess the effect of high temperature stress on yield forming traits, pollen viability, germination, oil, protein and glucosinolate and fatty acid profiles.

## Materials and Methods

The experiment was conducted at the Department of Genetics and Plant Breeding laboratory of Sher-e-Bangla Agricultural University, Bangladesh during 2020. All plants were grown in a controlled environment chamber at $22/17^0$C day/night temperature until the high temperature treatments were imposed. High temperature was imposed in a growth cabinet for 7 days during at early flowering and early siliqua formation stage. After imposing temperature stress, plants were return to $22/17^0$C growth chambers. Five temperature were used in this experiment : (i) control = continuous $22/17^0$C; (ii) $28/17^0$C at early flowering stage; (iii) $28/17^0$C at early siliqua formation stage; (iv) $35/17^0$C at early flowering stage; and (v) $35/17^0$C at early siliqua formation stage. The seeds of five genotypes (BARI Sarisha-6, Tori 7, Kallayania, BARI Sarisha-9 and BINA Sarisha-4) were used. Data were collected on shoot dry matter, seed yield, fertile pods, seed per pod and 1000 seed weight. In another experiment, more than 100 inflorescences for each genotype were randomly cut and immediately brought to the laboratory. Pollen grain from a single genotype were collected, mixed and immediately stored at $-20^0$C.

Method described by Singh *et al.* (2008) was used to check the pollen viability (PV) and pollen germination (PG). With the help of a clean, fine bristle paint brush, a homogenous layer of pollen grains were spread on the surface of the germination medium in a petri plate and transferred to the incubator at the respective temperature treatments: (i) $15^0$C; (ii) $20^0$C (iii) $25^0$C (iv) $30^0$C and (v) $35^0$C . To analyse the oil, protein and fatty acid, when the plants had flowered and siliquae set properly, all the plant were transferred to a growth chamber and exposed to a moderately high temperature treatment for a short period of 9 d following a day of acclimation to make 10d of treatment. During the acclimation, $25^0$C/$20^0$C temperature was applied. For high temperature treatment, a shorter period of 4d of very high temperature ($28^0$C/$23^0$C) following a day of acclimation to make 5d of treatment was applied. Similar temperature was applied during the acclimation day as that of the previous treatment. For control treatment, plants were kept at $22^0$C/$17^0$C temperature. Oil and protein concentration was determined by a previously described method (Young *et al.,* 2004). Glucosinolates was calculated by using the method described by Mailer and Pratley (1990). Fatty acid composition of the oil (oleic, linoleic, linolenic, palmitic and stearic acids) was determined by gas chromatography as adapted from Ayton *et al.,* (2001) and Mailer *et al.,* (2002).

## Results and Discussion

### Effect of temperature stress on dry matter, seed yield, fertile siliquae, seeds per siliqua and thousand seed weight in *Brassica rapa*

Shoot dry matter accumulation varied with temperature and genotypes. The temperature 28/$17^0$C had no influence on shoot dry matter accumulation however; increased temperature to 35/$17^0$C showed decreased shoot dry matter accumulation (Table 1). The response varied between two growth stages, early flowering was more sensitive to 35/$17^0$C (shoot dry matter decrease) compared to early siliqua stage. Seed yield of the main shoot of all genotypes was reduced on 35/$17^0$C at early flowering stage relative to the control while yield increased with 28/$17^0$C at early flowering stage. This suggests that the optimum daytime temperature for seed yield of the main shoot is closer to $28^0$C. The temperature treatment (28/$17^0$C) produced more siliqua during early flowering stage compared to control. However, temperature stress (35/$17^0$C) during early flowering stage produced less number of siliquae (Table 1). For temperature treatment 28/$17^0$C and 35/$17^0$C, did not produce difference in the genotypes during early siliqua formation stage (Table 1). This indicated that a short exposure of extreme temperature at a sensitive stage could be critical for crop yield as a mild temperature stress over a longer period. Among the genotypes, the largest seeds were produced by the 22/$17^0$C (control), but similar to at 28/$17^0$C treatment during early flowering stage. On the other hand, severe temperature stress (35/$17^0$C) at early flowering stage tended to reduce seeds per siliqua (Table 1).

**Table 1.** Mean plant dry matter, seed yield, fertile siliquae, seed per siliqua and thousand seed weight for temperature treatments in five *Brassica* genotypes

| Treatment | BARI Sarisha-6 | Tori 7 | Kallayania | BARI Sarisha -9 | BINA Sarisha-4 | Mean |
|---|---|---|---|---|---|---|
| | | | Shoot dry matter (g/plant) | | | |
| 22/17°C (control) | 22.15 | 20.83 | 24.91 | 24.3 | 23.8 | 23.19 |
| 28/17°C @ early flower | 21.7 | 22.55 | 20.2 | 22.8 | 21.72 | 21.79 |
| 28/17°C @ early siliqua | 19.43 | 18.28 | 21.3 | 19.66 | 18.93 | 19.52 |
| 35/17°C @ early flower | 18.2 | 18.32 | 22.44 | 21.83 | 17.73 | 19.70 |
| 35/17°C @ early siliqua | 18.79 | 19.03 | 22.67 | 20.55 | 20.45 | 20.29 |
| Mean | 20.05 | 19.80 | 22.30 | 21.82 | 20.52 | |
| LSD (T) and LSD (G) | | | 1.56 and 1.45 | | | |
| | | | Seed yield (g/plant) | | | |
| 22/17°C (control) | 0.74 | 0.63 | 1.68 | 1.75 | 0.94 | 1.14 |
| 28/17°C @ early flower | 1.83 | 1.72 | 1.25 | 1 | 1.65 | 1.49 |
| 28/17°C @ early siliqua | 0.71 | 0.62 | 0.83 | 0.84 | 0.81 | 0.76 |
| 35/17°C @ early flower | 0.88 | 0.2 | 0.13 | 0.25 | 0.18 | 0.32 |
| 35/17°C @ early siliqua | 0.86 | 1.04 | 0.83 | 0.9 | 0.79 | 0.88 |
| Mean | 1.00 | 0.84 | 0.94 | 0.94 | 0.87 | |
| LSD (T) and LSD (G) | | | 0.61 and 0.49 | | | |
| | | | Fertile siliqua /main stem | | | |
| 22/17°C (control) | 40 | 33 | 43 | 38 | 41 | 39.0 |
| 28/17°C @ early flower | 75 | 58 | 48 | 35 | 37 | 50.6 |
| 28/17°C @ early siliqua | 38 | 29 | 29 | 18 | 22 | 27.2 |
| 35/17°C @ early flower | 20 | 15 | 19 | 10 | 8 | 14.4 |
| 35/17°C @ early siliqua | 48 | 24 | 30 | 28 | 40 | 34.0 |
| Mean | 44.2 | 31.8 | 33.8 | 25.8 | 29.6 | |
| LSD (T) and LSD (G) | | | 6.0 and 7.0 | | | |
| | | | Seeds/ siliqua | | | |
| 22/17°C (control) | 27 | 16 | 18 | 16 | 12 | 17.8 |
| 28/17°C @ early flower | 22 | 12 | 13 | 13 | 10 | 14 |
| 28/17°C @ early pod | 10 | 15 | 12 | 9 | 7 | 10.6 |
| 35/17°C @ early flower | 15 | 7 | 10 | 12 | 11 | 11.0 |
| 35/17°C @ early siliqua | 12 | 11 | 9 | 14 | 5 | 10.2 |
| Mean | 17.2 | 12.2 | 12.4 | 12.8 | 9.0 | |
| LSD (T) and LSD (G) | | | 2.2 and 1.98 | | | |

**Table 1.** Contd.

| Treatment | BARI Sarisha-6 | Tori 7 | Kallayania | BARI Sarisha -9 | BINA Sarisha-4 | Mean |
|---|---|---|---|---|---|---|
| | | | 1000 Seed weight (g) | | | |
| 22/17°C (control) | 3.33 | 1.8 | 4.28 | 2.73 | 2.88 | 3.00 |
| 28/17°C @ early flower | 2.95 | 1.52 | 3.95 | 2.52 | 2.71 | 2.73 |
| 28/17°C @ early siliqua | 3.13 | 1.75 | 3.98 | 2.66 | 3.1 | 2.92 |
| 35/17°C @ early flower | 1.51 | 0.78 | 1.44 | 1.08 | 1.07 | 1.17 |
| 35/17°C @ early siliqua | 1.11 | 0.46 | 1.08 | 0.67 | 0.84 | 0.83 |
| Mean | 2.40 | 1.26 | 2.94 | 1.93 | 2.12 | |
| LSD (T) and LSD (G) | | | 0.54 and 0.42 | | | |

All the genotypes produced a lower seeds at moderate ($28/15^0$C) to high temperature stress ($35/17^0$C) at early siliqua formation stage. Furthermore, high temperature stress ($35/17^0$C) at early flowering stage and at early siliqua formation stage reduced 1000 seed weight in all genotypes. The temperature stress affected the yield forming traits for all the genotypes. High heat stresses have direct effect on flowering (Tayo and Morgan 1975; Angadi *et al.,* 2000) to support the plant photosynthetically. Therefore, fertile siliqua only developed from early flowers while the later flowers may not capable to form fertile siliqua. Previous report showed reduced seed weight under shorter exposure to high temperature stress in canola (Aksouh *et al.,* 2001) and declination in the duration of grain filling in cereals (Sofield *et al.,* 1977). Yield loss due to temperature stress has been reported in many crops including broccoli (Heather *et al.,* 1992), peanut (Vara Prasad *et al.,* 1999) and pea (Guilioni *et al.,* 1997).

## Effect of temperature stress on pollen viability and germination

To test the effect of temperature on pollen viability and germination, pollen were grown in solid pollen germination medium. A pollen grain was considered to be germinated when the pollen tube length exceeded or equalled the grain diameter. Maximum pollen viability and germination were recorded at $20^0$C followed by $25^0$C and $30^0$C [Fig. 1 (A and B)]. The lowest pollen viability and germination was recorded during high temperature stress ($35^0$C). Maximum pollen viability (96%) and germination (58%) were recorded at $20^0$C by Kallayania followed by Tori 7 (83% and 48% respectively) at $25^0$C. However, the temperature above $30^0$C reduces pollen viability and germination for all the genotypes (22% and 9% respectively). Reduced pollen viability and germination was observed by imposing increasing temperature in *Brassica rapa* genotypes. This might be due to the changes of proteins and lipids on the pollen coat (Lahlali *et al.,* 2014), loss of membrane integrity (Jain and Shivanna, 1989) and sugar presents in pollen grains (Pressman *et al*., 2002).

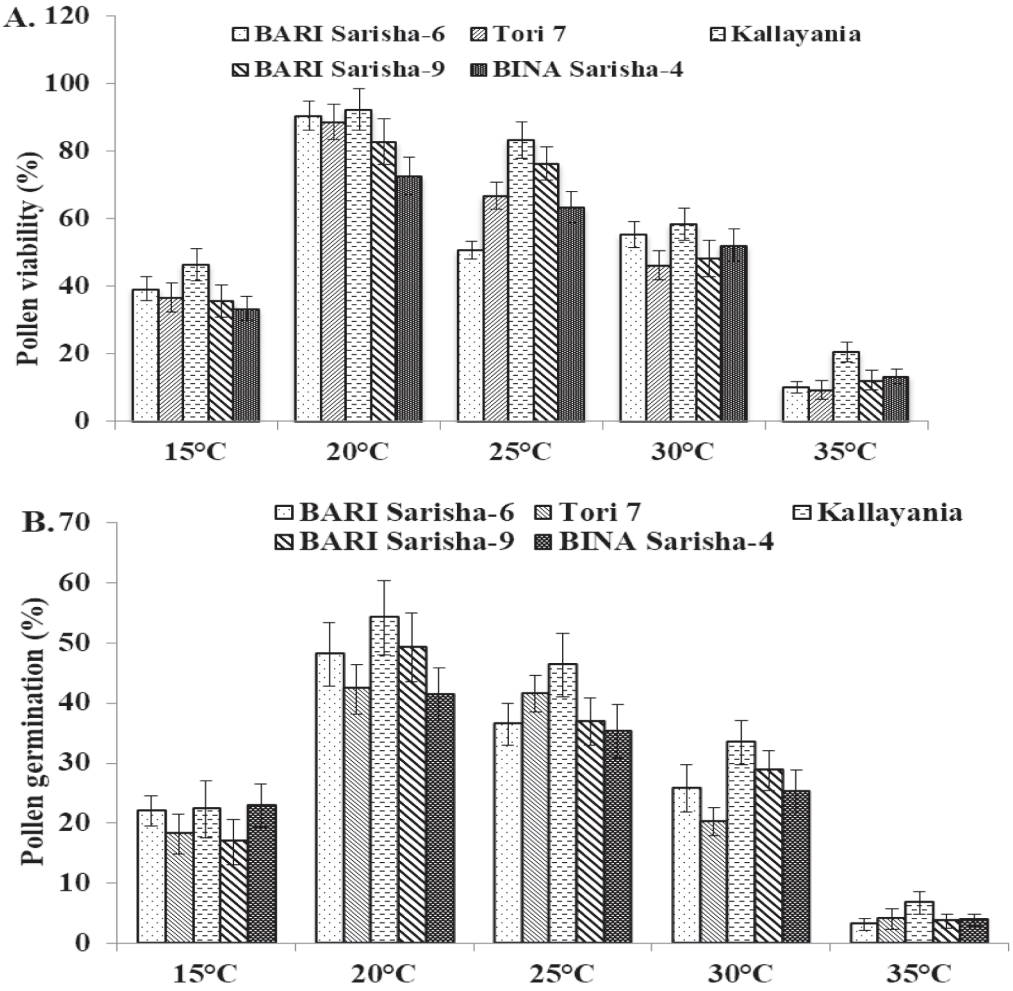


**Fig. 1.** Effect of temperature stress on pollen viability (PV) and pollen germination (PG) in five *Brassica* genotypes. A. Effect on pollen viability (PV) and B. Effect on pollen germination (PG). Error bars denote for ± SE of 15 measurements.

## Effect of temperature stress on oil, protein and glucosinolate concentration in *Brassica* genotypes

Out of the five genotypes, Tori 7 had the highest average oil concentration in the control (Table 2). The temperature stress (25/22$^0$C) imposed for 10d increased the oil and protein in BARI Sarisha-6 (Table 2). Very high temperature stress imposed for 5d decreased the oil concentration only in BARI Sarisha-9 and did not influence the protein fraction. Thus, very high temperatures exposure for 5d reduced the average oil and increased protein concentrations (Table 2). The result showed that high temperatures stress reduces the oil concentration and increase the protein concentration for all the

genotypes. Previous report also showed that the moderately high temperature treatment increased the oil/protein ratio in Canola (Aksouh-Harradj *et al.,* 2006). Strong negative correlation exists between oil and protein with high temperatures which might be explained by an increase of grain nitrogen (Aksouh-Harradj *et al.,* 2006) due to suppression of starch synthesis rather than to a change in the nitrogen quantity (Bullar and Jenner, 1985). High temperature alter protein biosynthetic pathway along with nitrogen concentration in the grain that changes different protein composition (Aksouh-Harradj *et al.,* 2006). Glucosinolate concentration differed between genotypes exposed to $25/22^0$C and $28/23^0$C temperature respectively (Table 2). High glucosinolate also reduces the feeding value of the rape (Mendham and Salisbury, 1995). In this context, genotype BARI Sarisha-9 showed low glucosinolate and much higher in Tori-7. Therefore breeding lines with low glucosinolate level need to be developed for future breeding program. Temperature stress during seed filling altered the chemical nature of *Brassica* oil especially in the unsaturated fatty acid.

**Table 2.** Effect of temperature stress on the oil, protein and glucosinolate concentration in five *Brassica* genotypes

| Treatment | BARI Sarisha-6 | Tori 7 | Kallayania | BARI Sarisha-9 | BINA Sarisha-4 | Mean |
|---|---|---|---|---|---|---|
| | | | Oil concentration (%) | | | |
| 22/17°C (control) | 38.8 | 40.33 | 39.25 | 38.18 | 37.88 | 38.88 |
| 25/20°C | 36.9 | 34.15 | 35.36 | 36.48 | 33.4 | 35.25 |
| 28/23°C | 32.2 | 31.23 | 32.48 | 30.55 | 32.66 | 31.82 |
| Mean | 35.96 | 35.23 | 35.69 | 35.07 | 34.64 | 35.32 |
| LSD (T) and LSD (G) | | | 4.0 and 3.0 | | | |
| | | | Protein concentration (%) | | | |
| 22/17°C (control) | 45.68 | 44.54 | 43.95 | 41.33 | 44.88 | 44.07 |
| 25/20°C | 44.25 | 40.22 | 41.35 | 42.68 | 40.83 | 41.86 |
| 28/23°C | 42.23 | 43.22 | 41.82 | 40.96 | 43.68 | 42.38 |
| Mean | 44.05 | 42.66 | 42.37 | 41.65 | 43.13 | 42.77 |
| LSD (T) and LSD (G) | | | 5.0 and 6.0 | | | |
| | | | Glucosinolate concentration (%) | | | |
| 22/17°C (control) | 22.22 | 30.5 | 19.8 | 11.52 | 17.65 | 20.33 |
| 25/20°C | 18.4 | 22.8 | 20.3 | 13.96 | 16.88 | 18.46 |
| 28/23°C | 11.4 | 31.8 | 18.4 | 9.64 | 20.33 | 18.31 |
| Mean | 17.34 | 28.36 | 19.5 | 11.70 | 18.28 | 19.04 |
| LSD (T) and LSD (G) | | | 2.98 and 1.87 | | | |

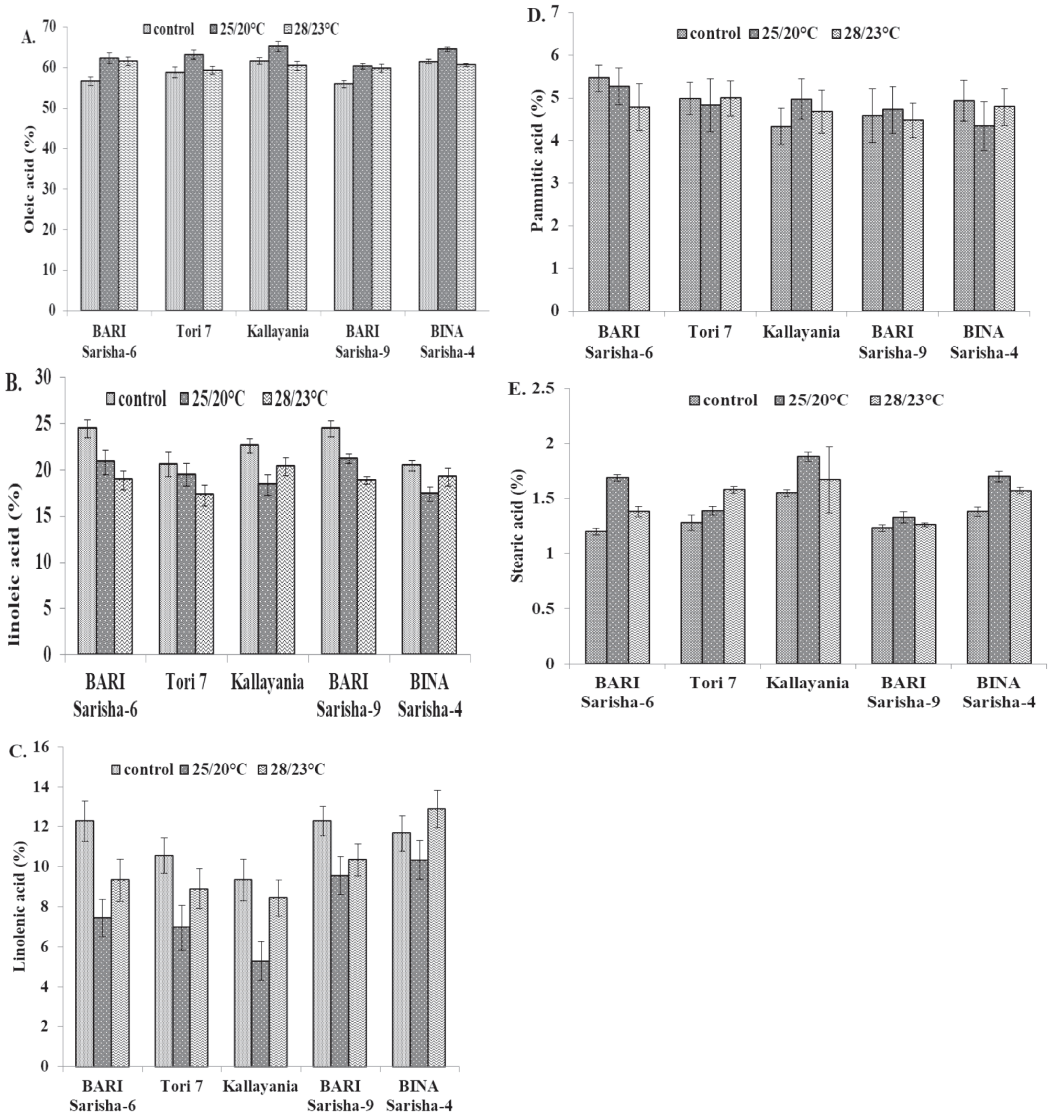


**Fig. 2.** Effect of temperature stress on the fatty acid composition in five *Brassica* genotypes. A. Effect on oleic acid, B. Effect on linoleic acid, C. Effect on linolenic acid, D. Effect on palmitic acid and E. Effect on stearic acid. Error bars denote the mean of at least 15 measurements ± SE.

## Effect of temperature stress on fatty acid profile in *Brassica* genotypes

No change was observed in oleic acid concentration in response to temperature stress (Fig. 2A) but linoleic acids concentration decreases with increasing the temperature (Fig. 2B) for all the genotypes. Plant exposed to $25/20^0$C temperature stress showed greatest changes in linolenic acid concentration (Fig. 2C). The lowest linolenic acid

concentration was observed by Kallayania (almost 5%) at 25/20$^0$C temperature stress while at 28/23$^0$C temperature BINA Sarisha-4 had highest (almost 13%) (Fig. 2C). The results showed that the levels of unsaturated fatty acids were elevated due to imposing temperature stress among the genotypes. This might be due to desaturase activities were restricted during high temperature. Similar reports in rapeseed and other species also showed that desaturase activities changes when plants exposed to high temperature (Gibson and Mullen, 1996, Aksouh-Harradj *et al.*, 2006). The saturated fatty acids level was similar for the genotypes in the control (Fig. 2D and E). No changes was observed in palmitic acid concentration (Fig. 2D) for all the genotypes, only stearic acid concentration increased by imposing the temperature stress (Fig. 2E). Similar finding were also showed that palmitic and stearic acids increases substantially by the elevated temperatures (Canvin, 1965; Percy, 1978; Green, 1986).

## Conclusion

Temperature stress not only had an effect on yield components but also effected oil concentration and fatty acid composition of *Brassica rapa* genotypes of Bangladesh. This could be a major issue in many regions of the country where high temperature combined with drought are common for cultivation of oilseeds. The optimum temperature for seed yield was closer to 28$^0$C. The temperature above 28$^0$C tended to reduce seed yield. The ideal temperature to get viable pollen and germination was 20$^0$C. The high temperature also affects oil and protein content for all the genotypes. BARI Sarisha-9 showed low glucosinolate level and could be used for future breeding program. The results of this experiment suggest a way for the breeders to select the genotypes that were affected by the high temperature.

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