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Full Length Research Paper

Effect of gibberrelic acid on α-amylase activity in heat stressed mung bean (*Vigna radiata* L.) seedlings

Simeen Mansoor* and Farzana N. Naqvi

Department of Genetics, University of Karachi, Karachi, Pakistan.

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High temperature is a serious threat that can alter the number of biochemical processes in plants, which may lead to reduce crop yield. Gibberellic acid (GA_3) is a plant growth hormone, responsible for growth, stress tolerance and regulation of many enzymes like amylase. Amylase is responsible for growth by hydrolyzing starch into maltose. This experiment was carried out to study the effect of GA_3 on α-amylase activity under heat stress conditions of four genotypes of mung bean (NM 19-19, NM 20-21, NM 121-123 and NCM 89). Seeds were sown in Petri dishes and incubated at different temperatures with and without 100 µM GA_3. It was found that the lethal temperature was 50°C when exposed for 2 h and reduced α-amylase activity in all genotypes which increased when a mild temperature (40°C,1 h) prior to lethal temperature was given. The application of GA_3 can further alleviate the effect of heat stress by increasing α-amylase activity. Genotypic variations was also observed and activity of amylase and heat stress tolerance index was highest in NM 19-19 and lowest in NM 20-21 for all treatments.

Key words: Mung bean, α-amylase, gibberellic acid, heat stress.

INTRODUCTION

Plant development is under the control of genes, profoundly affected by environment and number of stress conditions, where the most important is temperature (Scandalios, 1974). High temperature stress conditions can create a water deficit in plant tissues which in turn lead to injury of cell membranes and reduction in rate of transpiration, protein synthesis, enzymes and ion uptake and transport (Khalil et al., 2009). High temperature causes reduction in shoot dry mass, growth and net assimilation rates in number of plants (Wahid et al., 2007). Plants have evolved strategies like modulation of different enzymes (Thind et al., 1997) for preventing damage caused by rapid changes in temperature (Senthil-Kumar et al., 2007). Plants are able to survive, respond to, and acclimatize to mild temperatures which allow them to survive lethal temperature (Senthil-Kumar et al., 2007). α-Amylase is an enzyme which is involved in the hydrolysis of starch into maltose and is under nuclear gene control (Scandalios, 1974). It is responsible for active growth, and its activity increased in all plant organs with the progress of mung bean seedling growth (Kaur et al., 2001). Lethal temperature extremely retards seedling growth as well as amylase activity in winter wheat (Sultana et al., 2000). The exogenous application of GA_3 during heat stress is able to increase growth and amylase activity (Mitsui and Akazawa, 1986; Cavusoglu and Kudret, 2007). High temperature reduces the endogenous levels of GA_3 and its exogenous application counteracts the reduction in the endogenous level that was inhibiting the growth and related enzymes (Yakushikina and Tarasov, 1982; Kabar and Baltepe, 1990). Gibberellic acid was considered to promote seed germination; acting after abscisic acid (ABA)-mediated inhibition of germination has been overcome (Jacobsen et al., 2002).

The purpose of this study was to observe the effect of high temperature on α-amylase activity in the growth mung bean seedlings and to analyze the impact of GA_3 over α-amylase activity.

MATERIALS AND METHODS

Seeds of four mung bean genotypes (NM 19-19, NM 20-21, NM 121-123 and NCM 89) were obtained from National Agricultural

*Corresponding author. E-mail: simeenm@uok.edu.pk.

Table 1. Mean sum of squares of amylase activity (µg maltose/mg protein/5 min) in mung bean (*Vigna radiate.*L) seedlings harvested after temperature and GA_3 treatments.

Sources of variation	df	MS			
		0 h	24 h	48 h	72 h
Temperature (T)	5	790.71**	518.5**	930.1**	2945.1**
Genotype (G)	3	339.04**	341.17**	600.5**	1583.8**
T × G	15	10.81^{ns}	5.94^{ns}	23.6^{ns}	24.77^{ns}
Error	48	9.38	9.46	9.97	20.08

MS, Mean sum of squares; df, degree of freedom; ** highly significant; ns, non significant.

Research Centre (NARC), Islamabad, Pakistan and imbibed in distilled water (d/w) for 5 h then sterilized with 1% sodium hypochlorite solution for 5 min, rinsed many times with d/w. Sterilized seeds were sown in Petri dishes, lined with filter paper moistened with d/w and incubated at 30°C for 24 h. Seedlings were then incubated in 1% sucrose solution in 1 mM phosphate buffer and temperature treatments were given with and without 100 µM GA_3, according to the method of Chen et al. (1986) with minor modifications. Seedlings were harvested at 0, 24, 48 and 72 h after treatments and saved for biochemical analysis. Protein was estimated by the method of Lowry et al. (1951); amylase activity was estimated by Chrispeels and Varner (1967).

Heat stress tolerance index (HSTI) was calculated by the method of Porch (2006) using the following formula:

$$HSTI = (Yp) - (Ys) / (X\bar{p})^2$$

Where, YP = observation under unstressed condition, Ys = observations under stressed condition, and $X\bar{p}$ = mean of all genotypes under unstressed condition.

Statistical analysis

Analysis of variance was performed as two factorial in completely randomized design (CRD) with three replications by computer software SPSS version 13. Duncan's multiple range test was performed at $P \leq 0.05$ level of significance for mean comparisons (Steel and Torrie, 1980).

RESULTS

Mean sum of square for amylase activity (µg maltose/mg protein/5 min) in mung bean seedlings harvested 0, 24, 48 and 72 h after temperature and GA_3 treatments showed highly significant differences for temperature and genotypes with non significant interaction for all harvests (Table 1).

Figure 1a shows that NM 19-19 exhibited increased α-amylase activity as the growth proceeded. Its activity was significantly reduced at lethal temperature (50°C, 2 h), significantly enhanced when pretreatment of mild temperature (40°C,1 h) was given prior to lethal temperature (30°C +40°C +50°C) for all harvests. Amylase activity was significantly higher when GA_3 was applied during 30°C (30°C GA_3) as compared to 30°C for all harvests; it means that application of GA_3 was responsible for the further rise in amylase activity during unstressed condition. It was also enhanced by the application of GA_3 under stressed conditions. GA_3 was applied during pretreatment as 30°C + 40°C (GA_3) + 50°C and as 30°C + 40°C + 50°C (GA_3), showing more promotion in 30°C + 40°C + 50°C (GA_3), but non significantly for all harvests. This pattern was same for all other genotypes, but more promotion in GA_3 treated samples and less inhibition in temperature stressed samples was seen in NM 19-19.

Similarly, HSTI when calculated for amylase activity for all treatments and genotypes(Table 2), resulted in highest HSTI, found HSTI highest when GA_3 was applied during unstressed condition [30°C (GA_3)], reduced in seedlings exposed to 40°C prior to 50°C, and lowest was seen at lethal temperature stress (50°C). More HSTI means more tolerance (Table 2). Genotypic variations with respect to amylase activity was also detected. NM 19-19 performed well amongst all genotypes as far as amylase activity was concerned (Figures 1a, b, c and d).

DISCUSSION

The present results reveal that amylase activity increased as the growth of seedlings was proceeded for all genotypes and treatments. Kaur et al. (2001) reported that with the progress of seedlings growth of mung bean, amylolytic activity increased in all organs like roots, shoots and cotyledons to hydrolyze starch.

Current observed great reduction in mean α-amylase activity when seedlings were exposed to lethal temperature of 50°C for 2 h, but this decrease in α-amylase activity was somewhat improved when a pretreatment of 40°C (1 h) before lethal temperature was given. Sultana et al. (2000), reported the effect of various temperatures on winter wheat seeds and observed that α-amylase expression during germination was maximum at 29°C, although a high temperature of 38°C prevented the synthesis of α-amylase. Our results are also supported by the findings of Johnston et al. (2002) who reported that heat shock of 40°C induced the synthesis of heat shock proteins (HSPs) and suppresses the synthesis of α-amylase in barley aleurone, however if samples were treated with an increase of 2.5°C every 30 min from the normal temperature of 25°C to the heat shock temperature

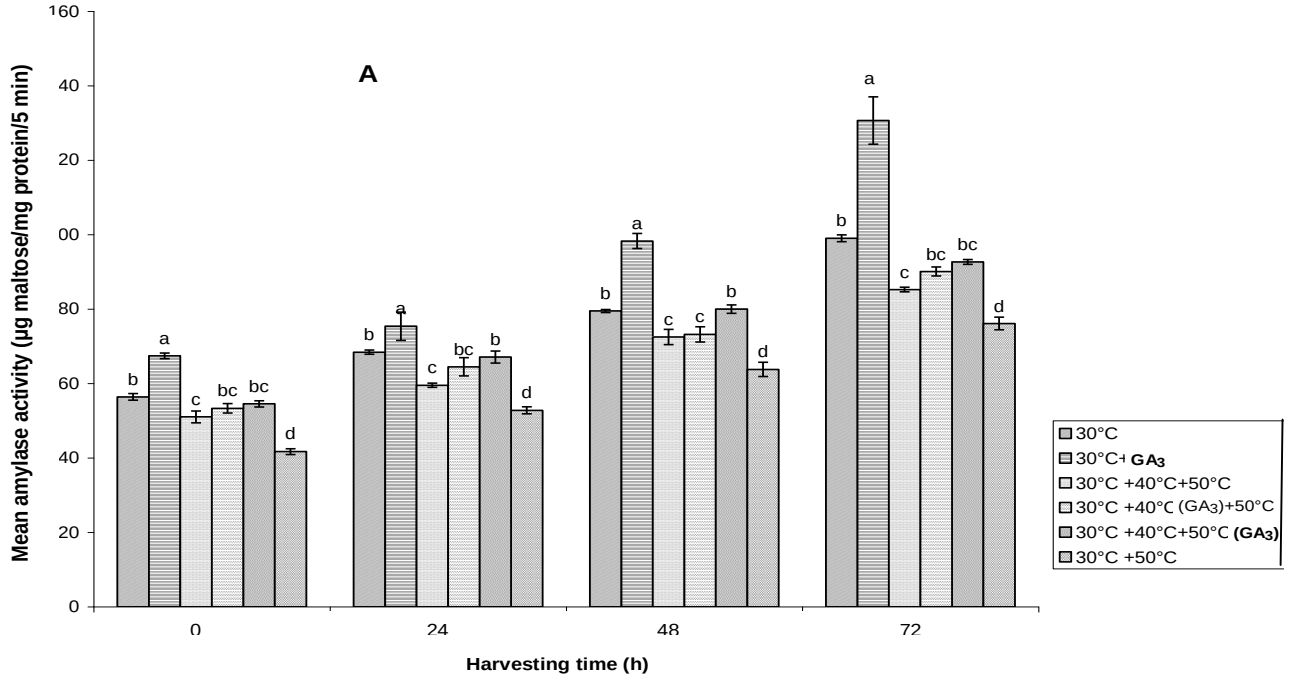

Figure 1a. Amylase activity (µg maltose/mg protein/5 min) in mung bean (*Vigna radiata*) seedlings of genotype NM 19-19, harvested after temperature and GA_3 treatments. Error bars represent ± SE of three replications. Similar alphabets indicate non significant difference ($p \leq 0.05$) among treatments of each harvest.

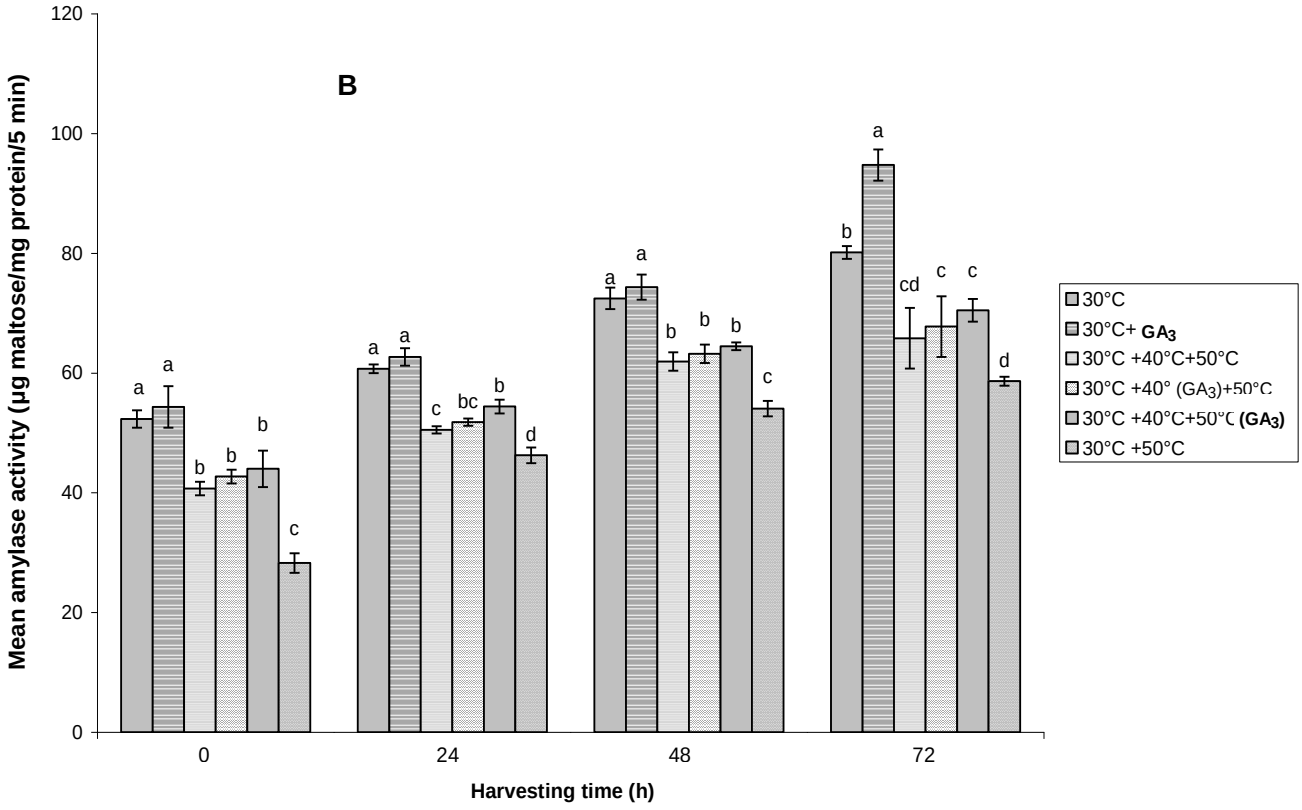

Figure 1b. Amylase activity (µg maltose/mg protein/5 min) in mung bean (*Vigna radiata*) seedlings of genotype NM 20-21, harvested after temperature and GA_3 treatments. Error bars represent ±SE of three replications. Similar alphabets indicate non significant difference ($p \leq 0.05$) among treatments of each harvest.

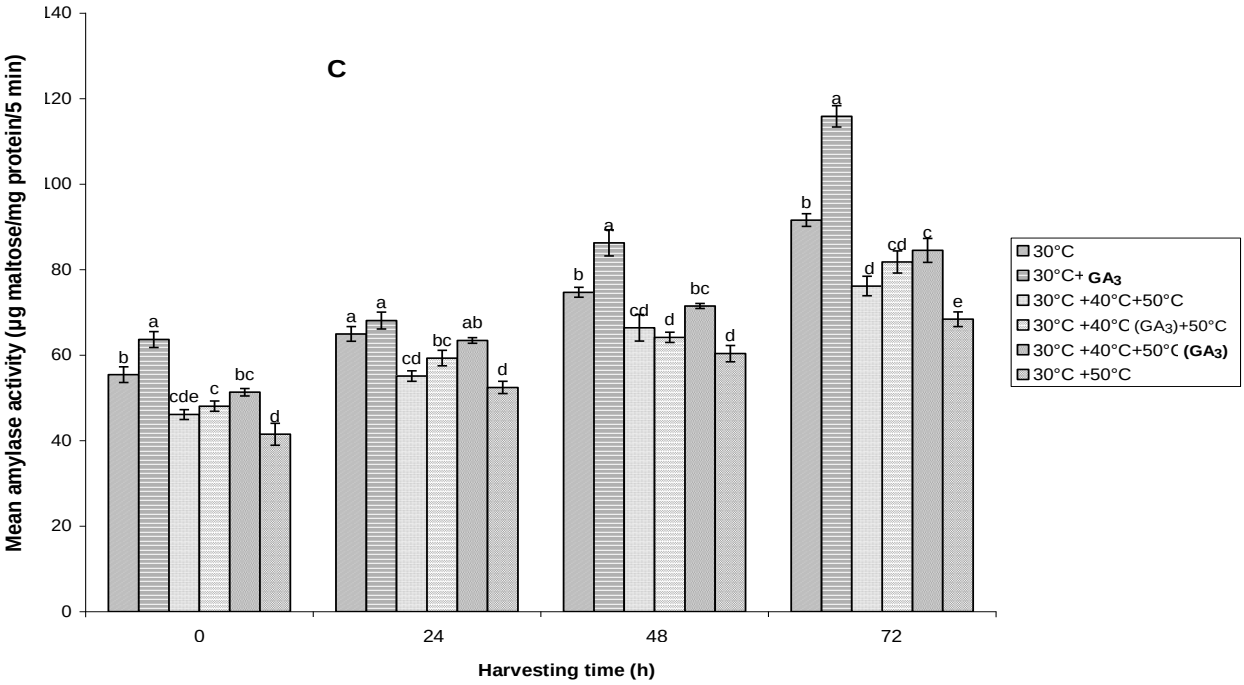

Figure 1c. Amylase activity (µg maltose/mg protein/5 min) in mung bean (*Vigna radiata*) seedlings of genotype NM 121-123 , harvested after temperature and GA_3 treatments. Error bars represent ±SE of three replications. Similar alphabets indicate non significant difference (p≤0.05) among treatments of each harvest.

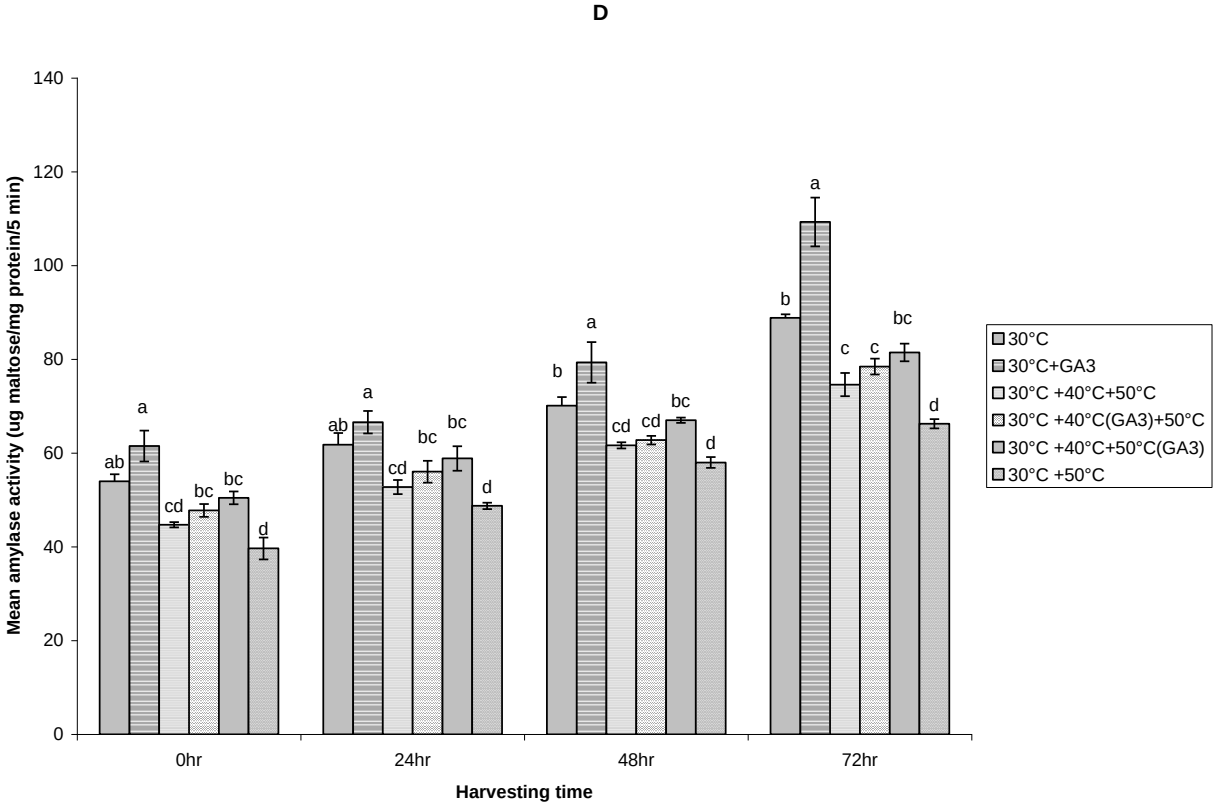

Figure 1d. Amylase activity (µg maltose/mg protein/5 min) in mung bean (*Vigna radiata*) seedlings NCM 89 , harvested after temperature and GA_3 treatments. Error bars represent ±SE of three replications. Similar alphabets indicate non significant difference (p≤0.05) among treatments of each harvest

Table 2. Analysis of heat stress tolerance (HST) index for mean amylase activity for three trials in mung bean seedlings under temperature and GA_3 treatments.

Genotype	Treatment	0 h	24 h	48 h	72 h
NM 19-19	30°C(GA_3)	0.079	0.078	0.088	0.099
	30°C + 40°C + 50°C	0.060	0.062	0.065	0.065
	30°C + 40°C(GA_3) + 50°C	0.063	0.067	0.066	0.068
	30°C + 40°C + 50°C(GA_3)	0.064	0.070	0.072	0.070
	30°C + 50°C	0.049	0.055	0.057	0.073
NM 20-21	30°C(GA_3)	0.059	0.058	0.061	0.058
	30°C + 40°C + 50°C	0.044	0.046	0.050	0.040
	30°C + 40°C(GA_3) + 50°C	0.046	0.048	0.052	0.041
	30°C + 40°C + 50°C(GA_3)	0.048	0.050	0.053	0.043
	30°C + 50°C	0.031	0.042	0.044	0.036
NM 121-123	30°C(GA_3)	0.074	0.067	0.073	0.081
	30°C + 40°C + 50°C	0.053	0.054	0.056	0.053
	30°C + 40°C(GA_3) + 50°C	0.055	0.058	0.057	0.057
	30°C + 40°C + 50°C(GA_3)	0.059	0.062	0.060	0.059
	30°C + 50°C	0.048	0.052	0.051	0.048
NCM 89	30°C(GA_3)	0.069	0.062	0.063	0.075
	30°C + 40°C + 50°C	0.050	0.049	0.049	0.051
	30°C + 40°C(GA_3) + 50°C	0.054	0.052	0.0499	0.053
	30°C + 40°C + 50°C(GA_3)	0.057	0.055	0.053	0.055
	30°C + 50°C	0.045	0.045	0.046	0.045

of 40°C, synthesis of α-amylase would be over five folds greater than the heat shocked sample. Therefore the rapidity of the onset of the heat stress appears to be important for plant to acclimatize to high temperature stress in the field.

Present research showed that the activity of amylase was reduced under lethal temperature stress, however the mild temperature treatment prior to lethal temperature treatment induce amylase activity. Exogenous application of GA_3 was able to further induce α-amylase activity during temperature stressed as well as unstressed conditions therefore it is suggested that by the deficiency of amylase there could be the unavailability of maltose which is prerequisite of growth. It is supported by Zeid, (2011), who reported that amylase play an important role in germination by providing starch hydrolyzate, which was decreased due to stress and the deficiency in amylase and maltose was overcome by GA_3 treatment. Gibberellic acid can reduce the adverse effect of high temperature stress during seed germination (Cavusoglu and Kudret, 2007). It is reported that high temperature reduces the endogenous levels of cytokinins, gibberellins and auxins (Kabar and Baltepe, 1990). It is possible that exogenous GA3 counteracts the reduction in the endogenous levels of plant hormones that promote growth and increase endogenous level of plant hormones. Toyomasu et al. (1994) reported that exogenous GA3 promotes germination with a reduction in endogenous ABA content.

Heat stress indices have been developed for the evaluation of high temperature stress in plants, stress tolerance index (HST) has been used for comparing genotypic performance across years to environment and used to identify genotypes that perform well under both stress and non stress conditions (Fernandez 1993; Porch 2006). It is concluded that by the pretreatment of GA_3, thermotolerance can further be raised in all four mung bean genotypes, however the HSTI and amylase activity in NM 19-19 was highest and lowest in NM 20-21 (Figures 1a and b). Therefore by looking at the data, it may be suggested that NM 19-19 was considered to be heat tolerant and NM 20-21, as heat sensitive genotype.

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