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RESEARCH ARTICLE

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Effect of plant growth regulators on two different types of eggplant flowers regarding style length and fruit setting

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Abstract

Aim of study: (i) to explore differences between eggplant flowers capable of setting fruit including long (LGs) and medium style flowers (MEs) and those which suffer from severe problems with fertility and fruit setting including short style ones (SRTs); (ii) to study the effect of plant growth regulators on floral morphology and fruit setting.

Area of study: Isfahan University of Technology, Isfahan, Iran, 2017 and 2018.

Material and methods: First, the floral morphology and initial fruit setting of 13 eggplant genotypes from Iran were investigated. Then the differences between LGs and SRTs of two genotypes were explored. Finally, the effect of 1-naphthaleneacetic acid (NAA) and spermidine (Spd) on floral morphology and initial and final fruit setting of these two genotypes was determined.

Main results: Results showed SRTs were not capable of fruit setting. Compared to SRTs, LGs had larger central canals, higher protein, total sugar, reducing sugar and K concentrations, as well as longer polar axis and pollen tubes and greater pollen viability. Although 1.5 mM Spd and 20 mg L^{-1} NAA resulted in increasing of LGs and MEs, and also total initial fruit set, surprisingly, no significant differences were observed in the final yield and final fruit set between the control and these treatments.

Research highlights: Since the rate of fruit dropping was higher in those treatments compared to the control, plants with more SRTs likely regulate their final load by abscising their flowers, and plants with more LGs regulate them by abscising their fruits.

Additional key words: auxin; heterostyly; nutrient concentration; polyamine; *Solanum melongena*; yield

Abbreviations used: IAA (indole acetic acid); IKI (iodine + potassium iodide); LG (long style flower); ME (medium style flower); NAA (1-naphthaleneacetic acid); PGR (plant growth regulator); Put (putrescine); Spd (spermidine); Spm (spermine); SRT (short style flower); TTC (2,3,5-triphenyl tetrazolium chloride).

Authors' contributions: Designed the experiment: SKH, BB and MM. Performed the experiments; analyzed and interpreted the data; drafted of the manuscript: SKH. Supervised the work: MM and BB. Technical support, revised the manuscript: BB, MM and MHE.

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Introduction

The eggplant, or brinjal (*Solanum melongena* L.), is an important Solanaceae crop from the tropical and subtropical regions of India as its primary centre of origin (Meyer *et al.*, 2012). It is an economically important crop throughout the world (Caruso *et al.*, 2017), with global annual production surpassing 54 million tons in 2018. With an annual production of 666,838 tons, Iran is the fourth leading eggplant producer after China, India, and Turkey (Faostat, 2018). Eggplant flowers are large and usually violet-colored. They consist of five united and persistent sepals, five united and cup-shaped petals, five stamens alternating with the corolla, united carpels, and superior ovaries arranged either solitary or in inflorescence (Rashid & Singh, 2000; Hazra *et al.*, 2003; Jagatheeswari, 2014). As a heterostylous species, eggplant flowers are classified into the three different kinds of flowers: long style (LG), medium style (ME), and short style (SRT) depending on their style length relative to that of the stamen (Handique & Sarma, 1995; Sękara & Bieniasz, 2008). Studies on the relationship between fruit set and stigma position have reported a larger fruit set in flowers in which the stigma is positioned above or at the same level as the anther tip, while severe fertility and fruit set problems have been observed in SRT ones (Prasad & Prakash, 1968; Passam & Bolmatis, 1997; Srinivas *et al.*, 2016; Pohl *et al.*, 2019). Since a considerable portion

of eggplant flowers are SRT, their failure to set fruits decreases their fruit-yielding potential to a considerable extent (Chadha & Saimbhi, 1977). Although heterostyly in eggplant flowers is a varietal characteristic (Abney, 1997; Kowalska, 2006; Sękara & Bieniasz, 2008), it is affected by factors such as plant age, fruiting dynamics (Lenz, 1970), and environmental conditions (Sun *et al.*, 1990; Abney, 1997).

Plant growth regulators (PGRs) such as auxin (Woodward & Bartel, 2005) and polyamines (Borrell *et al.*, 1997) are essential for plant growth, and their exogenous application over the years has been reported to play important roles in controlling the flowering and fruit setting in many crops (Aliyu *et al.*, 2011; Choudhury *et al.*, 2013). It has been reported that SRTs treated with plant hormones could become LGs and MEs (Ravestijn, 1983). Few studies have focused on auxin's effects on heterostyly in the *Solanum* species. Foliar application of indole acetic acid (IAA) has been reported to increase the percentage of LGs in *Solanum khasianum* compared to the control plants, although no significant effects were observed on fruit setting (Ravindran, 1981). Moniruzzaman *et al.* (2015) reported that IAA, especially at concentration of 40 mg L^{-1}, significantly increased the percentages of LGs and MEs in eggplant. The effects of polyamines on flower gender and fertility have also been reported in the literature; however, the relationship between polyamines and heterostyly is still not clear. Various concentrations of polyamines have been reported in sterile and fertile organs of certain plant species (Liu *et al.*, 2006). The infertile flower lines in tobacco (Malmberg, 1980), maize (Martin-Tanguy *et al.*, 1979), stem mustard (Guo *et al.*, 2003), Araceae species (Ponchet *et al.*, 1980), and tomato (Rastogi & Sawhney, 1990a) have been observed to contain less polyamine than the fertile ones. However, Rastogi & Sawhney (1990b) reported higher levels of polyamines in male sterile tomato plants than in normal ones. This correlation indicates that polyamines might play a role in changing the heterostyly in eggplants.

To the best of the present authors' knowledge, there are few published reports on the differences between the two types (LG and SRT) of flowers in eggplants. The present study was therefore designed and implemented to determine the percentages of flowers capable (LGs + MEs) and incapable of setting fruits (SRTs), as well as fruit setting rates in these kinds of flowers in 13 eggplant genotypes from Iran. The authors also explored the differences in morphological and micromorphological traits, as well as nutrient concentrations between the two types of flowers in two selected genotypes. In addition, the effects of different concentrations of spermidine (Spd) and 1-naphthaleneacetic acid (NAA) were compared not only on the percentage of LG, ME and SRT flowers as well as initial fruit set rates in solitary and inflorescence types, but also on the final yield and final fruit set of those two eggplant genotypes. We originally assumed if PGRs can increase the proportion of LGs to SRTs, the rate of initial fruit set will increase, and so the final yield and fruit set also will increase. To our knowledge, the effect of polyamine on the eggplant flowering based on style length and fruit setting is reported for the first time in the present study. Although the effect of Auxin has previously been studied by some researchers, they have only focused on the proportion of LGs to SRTs and initial fruit setting rate; no attempt was made to continue the research until the end of the growing season. In this study we explore the following question: does increasing the proportion of LGs to SRTs and initial fruit setting rate increase the final yield and final fruit setting of the eggplant?

Material and methods

First experiment

Plant material and experimental design. Thirteen eggplant genotypes obtained from the Gene Bank of the Agricultural Research Institute of Iran were used in this study (Table 1). In the middle of March 2017, 60 seeds from each genotype were sown into boxes filled with peat and perlite at a ratio of 4:1 v/v and placed inside a glass greenhouse. Six weeks after sowing, uniform seedlings were transplanted at 60 × 60 cm into an experimental field at Isfahan University of Technology, Isfahan, Iran (32° 42′ N; 51° 28′ E; 1624 m asl). The soil of the experimental site was sandy loam with a neutral pH suitable for cultivation. Prior to cultivation, the field was ploughed with 25 tones ha^{-1} of organic manure. The commercial fertilizer including NPK 20-20-20+B+Cu+Fe+Mn+Mo+Zn was also applied once a month at a concentration of 1 mg L^{-1} and the plants were irrigated using the drip irrigation method. Cultural practices and pest management were conducted according to standard recommendations during the growing season. The experiment was carried out in a randomized complete block design, with treatments arranged in a factorial scheme with three blocks. Each block included 13 genotypes, and each genotype included three plants.

Sampling and measurements. The plants were inspected daily to record the numbers of LG, ME, and SRT flowers, and each flower type was marked by a specific colour. Once the flowers set fruits, the number of fruits formed from each kind was counted and recorded. This procedure for data collection was continued throughout the reproductive stage from the beginning of June up to the end of August.

Second experiment

Plant material and experimental design. Based on their higher percentages of SRTs, the two genotypes 'TN74128' and 'TN74243' were chosen to study the likely differences between SG and LG flowers. This trial was conducted in 2018, with the same cultural conditions to last season. The experiments were conducted in a completely randomized design with two kinds of flowers (LG and SRT) and three replications.

Morphological traits measurement. Style, stamen, anther, and pedicel lengths as well as stigma width and pedicel diameter were measured using a caliper on the millimeter scale. Fresh weights of flower, pistil, ovary, and stigma were also measured using a sensitive digital scale and reported in milligrams.

Nutrient concentrations measurement. Style and stigma tissues were separated from both LGs and SRTs to determine their total sugar, reducing sugar, and protein concentrations. Total sugar concentration was measured using the anthrone method by spectrophotometry at 625 nm (McCready *et al.*, 1950), and reducing sugar concentration was determined using dinitrosalicylic acid by spectrophotometry at 575 nm according to the method described in Miller (1959). Finally, Bradford's method (Bradford, 1976) was used to determine protein concentration, and used the spectrophotometry to measure the absorbance at 595nm.

Mineral nutrients K, Ca, Mg, and B were measured in the style + stigma samples taken from both types (LG and SRT) of flower. The dry ashing method was used to extract K, Ca, and Mg in 2 mmol L^{-1} HCl (Chapman & Pratt, 1961; Waling *et al.*, 1989). K concentration in the extract was then determined using a Jenway PFP7 flame photometer, while those of Ca and Mg were determined by atomic absorption spectroscopy (Perkin-Elmer 3030). Finally, the azomethine-H colorimetric method was used to determine boron concentration (Carter, 1993).

Micromorphological traits measurement. Light microscopy was used to study differences between style tissues of LGs and SRTs. Style samples from the two types of flower were cut with a razor blade and stained using the double staining method (methyl green and carmine) before they were examined under an Olympus CH-2 microscope equipped with a digital camera.

Pollen grains were collected from both LGs and SRTs. The number of pollen grains in the anther was estimated according to the Dafni method (Carter, 1993), and pollen size was determined based on the grains' polar axis and equatorial diameter using software with the Olympus CH-2 microscope.

Pollen viability was determined using two TTC (2,3,5-triphenyl tetrazolium chloride) and IKI (iodine + potassium iodide) staining tests. A TTC concentration of 1% was used to determine viability after 2 hr (Nortin, 1966). The IKI medium consisted of 1 g potassium iodide and 0.5 g iodide dissolved in 100 mL of distilled water. Pollen viability was checked after 2 min of exposure to the medium (Bolat & Pirlak, 1999).

The *in vitro* pollen germination test was used to determine pollen germination percentages after 2, 4, 6, and 8 hr. For this purpose, a culture medium was made as described in Karni & Aloni (2002). This medium consisted of 100 g sucrose, 500 mg calcium nitrate, 120 mg magnesium sulphate, 100 mg potassium nitrate, and 120 mg boric acid dissolved in 1000 mL of deionized water, to which 10 g agar was added. A drop of this solution was poured onto each slide before fresh pollen samples were placed with a needle into the nutrient medium, and kept in Petri dishes lined with moist filter paper. The Petri dishes were then placed into the growth chamber at 25°C under a florescent

Table 1. List of Iranian *Solanum melongena* genotypes used in this study along with their sources.

Genotype	Origin	Fruit character	Longitude	Latitude	Altitude (m)
TN74237	Hormozgan	Dwarf type	56° 27' E	27° 18' N	21
TN74238	Hormozgan	Egg-shaped	56° 27' E	27° 18' N	30
TN74239	Hormozgan	Long and slender	56° 27' E	27° 18' N	30
TN74161	West Azarbayejan	Long and slender	54° 18' E	32° 33' N	325
TN74128	Kordestan	Dwarf type	46° 90' E	35° 61' N	555
TN74231	Kohgiluyeh	Long and slender	50° 40' E	30° 49' N	600
TN74197	Isfahan	Egg-shaped	51° 67' E	32° 65' N	900
TN74243	Kordestan	Long and slender	46° 90' E	35° 61' N	1000
TN74116	Khorasan	Long and slender	54° 18' E	32° 33' N	1100
TN74100	Ghazvin	Dwarf type	50° 01' E	36° 27' N	1280
TN74120	East Azarbayejan	Egg-shaped	54° 18' E	32° 33' N	1350
TN74250	Zanjan	Long and slender	48° 30' E	36° 41' N	1700
TN74156	Kerman	Egg-shaped	57° 07' E	30° 28' N	1756

light for observation under the Olympus microscope. Pollen tube growth was also measured after 8 hr.

Third experiment

Plant material and hormonal application. In 2018, a separate experiment, with the same cultural conditions to last season was conducted in a randomized complete block design with seven levels of plant growth regulators of Spd (0.5, 1, and 1.5 mM), NAA (20, 30, and 40 mg L^{-1}), and foliar-sprayed water as the control, in three replicates each including three plants. These treatments were exploited for two genotypes 'TN74128' and 'TN74243'. A sensitive electronic balance was used to weigh the PGRs. Solutions were prepared and poured into hand-held sprayers to be directly sprayed on the plants three times at four-week intervals beginning at the flowering onset (*i.e.*, six weeks after transplanting). The spraying was carried out early in the morning to avoid rapid desiccation of the spray solution due to transpiration.

Measurements. Observations of flower morphology and fruit setting were recorded twice a week from the beginning of the flowering stage through the reproductive stage. The data included the number of flowers with LG, ME, and SRTs formed both in solitary and in inflorescence, as well as the number of fruits formed from these three flower types as initial fruit set. Number of fruits per plant as final fruit set and yield per plant as well as fruit abscission rate were also determined.

Data analysis

The data collected were subjected to analysis of variance using the SAS software (vers. 9.1, SAS Inst., Cary, NC, USA) and SPSS software (vers. 19, IBM Corp, NY), and differences were compared using the least significant differences (LSD) test (p<0.05).

Results and discussion

Types of flowers and fruit setting based on style length

Except for 'TN74128' that contained almost the same percentages of the flower types, all the genotypes recorded greater percentages of LGs + MEs that are capable of fruit setting than SRTs (Table 2). Previous studies also revealed that among all types of eggplant flowers, LGs and MEs often occur in higher number than SRTs (Nagasawa *et al.*, 2001; Pohl *et al.*, 2019). However, the SRTs accounted for a considerable percentage of flowers on the plants (from about 20% to 45%).

A close relationship was established between fruit setting and style length in eggplant genotypes such that the LGs and MEs of all the genotypes investigated showed satisfactory fruit setting rates (42% to 76%), while only a small percentage (< 4%) of SRTs set fruits with most of their flowers aborted (Table 2). Pandit *et al.* (2010) and Pohl *et al.* (2019) also observed the higher percentage of

Table 2. Percentage of flowers based on style length and percentage of fruit setting from those flowers in 13 eggplant genotypes.

Genotype	Flower (%)		Fruit setting (%)	
	LG+ME	SRT	LG+ME	SRT
TN74237	57.3^{de}	33.46^{hi}	63.41^{c-e}	3.76^{i}
TN74238	63.64^{b-d}	27.43^{i-m}	54.7^{fg}	0.24^{i}
TN74239	41.64^{fg}	36.00^{gh}	70.38^{a-c}	0.67^{i}
TN74161	57.54^{de}	30.44^{h-k}	42.05^{h}	0.94^{i}
TN74128	43.60^{f}	45.50^{f}	61.73^{d-f}	3.12^{i}
TN74231	60.5^{cd}	24.54^{l-n}	50.80^{g}	1.24^{i}
TN74197	64.71^{bc}	25.09^{k-n}	72.14^{ab}	0.74^{i}
TN74243	52.07^{e}	32.46^{h-j}	67.48^{b-d}	1.70^{i}
TN74116	59.89^{cd}	29.06^{i-m}	66.29^{b-e}	2.19^{i}
TN74100	65.17^{bc}	26.07^{j-m}	57.44^{e-g}	2.03^{i}
TN74120	73.74^{a}	19.3^{1n}	76.50^{a}	0.0^{i}
TN74250	67.79^{ab}	23.33^{mn}	46.18^{h}	1.47^{i}
TN74156	64.67^{bc}	19.22^{n}	69.87^{a-d}	0.46^{i}

LG: long style flower. ME: medium style flower. SRT: short style flower. Values with the same letters between columns and rows for percentage of flowers and percentage of fruit setting have no significant difference using LSD test at 5% probability.

fruit setting from LGs and MEs respect to non-reproductive flowers (SRTs).

The question that arises is: which differences between LGs and SRTs could have induced fruit setting in the former? To answer this question, certain features of the two types of flowers were studied in the two genotypes chosen based on their high SRT percentages.

Features of LGs and SRTs

Morphological features

As expected, in both genotypes LGs exhibited not only substantially longer styles but also wider stigmas than SRT flowers did. Moreover, LGs recorded far greater pedicel diameters and lengths, as well as heavier flowers, pistils, ovaries, and stigmas (Table 3). In concordance with these results, Mohideen *et al.* (1977) found that higher fruit setting is associated with larger numbers of LGs, as they produce fleshy ovaries and thick pedicels. Kowalska (2006) reported lower stigma weights in SRTs and Salas *et al.* (2012) noted that SRTs had not only smaller ovaries but also smaller anthers and buds. Moreover, Sękara & Bieniasz (2008) observed that SRTs had smaller ovaries and stigmas.

Nutrient concentrations

Given that special nutrients and nutritional elements are needed for pollen grains to germinate on the stigma

Table 3. Differences between long (LG) and short style flowers (SRT) of two eggplant genotypes in morphological features, nutrient concentrations and micromorphological traits.

	TN74128		TN74243	
	LG	**SRT**	**LG**	**SRT**
Morphological features				
Style length (mm)	12.73[a]	2.60[b]	10.15[a]	1.92[b]
Stamen length (mm)	14.23[a]	11.76[b]	13.77[a]	13.01[a]
Stigma width (mm)	1.92[a]	0.88[b]	1.75[a]	0.80[b]
Pedicle diameter (mm)	2.76[a]	1.86[b]	2.86[a]	1.86[b]
Pedicle length (mm)	2.25[a]	1.16[b]	2.53[a]	1.83[b]
Flower weight (g)	0.76[a]	0.35[b]	0.74[a]	0.43[b]
Pistil weight (g)	0.18[a]	0.06[b]	0.17[a]	0.05[b]
Ovary weight (g)	0.16[a]	0.05[b]	0.15[a]	0.01[b]
Stigma weight (mg)	0.0032[a]	0.0001[b]	0.0016[a]	0.0002[b]
Nutrient concentrations				
Protein of style (µg mg^{-1})	0.89[a]	0.38[b]	0.82[a]	0.14[b]
Protein of stigma (µg mg^{-1})	1.48[a]	0.42[b]	0.78[a]	0.14[b]
Total sugar of style (µg mg^{-1})	9.30[a]	6.14[b]	18.30[a]	11.30[b]
Total sugar of stigma (µg mg^{-1})	9.01[a]	4.63[b]	20.86[a]	7.21[b]
Reducing sugar of style (µg mg^{-1})	11.57[a]	6.92[b]	9.33[a]	7.53[a]
Reducing sugar of stigma (µg mg^{-1})	14.81[a]	10.41[b]	13.57[a]	7.53[b]
K of style and stigma (µg mg^{-1})	26.97[a]	23.16[a]	28.13[a]	24.00[a]
Ca of style and stigma (µg mg^{-1})	4.39[a]	6.56[a]	2.17[b]	9.80[a]
Mg of style and stigma (µg mg^{-1})	2.85[b]	4.25[a]	3.18[a]	4.20[a]
B of style and stigma (µg mg^{-1})	1.33[b]	2.52[a]	0.57[b]	8.59[a]
Micromorphological traits				
Number of pollen per anther	13000[a]	12667[a]	25500[a]	18000[b]
Polar axis (µm)	16.21[a]	14.01[b]	15.71[a]	13.86[b]
Equatorial diameter (µm)	8.35[a]	8.29[a]	8.13[a]	8.10[a]
Pollen viability by TTC (%)	60.67[a]	39.50[b]	59.0[a]	35.0[b]
Pollen tube length (µm)	25.10[a]	14.20[b]	26.33[a]	15.80[b]

Values with the same letters in each row for each genotype have no significant difference using LSD test at 5% probability. TTC: 2,3,5-triphenyl tetrazolium chloride

surface and for pollen tubes to grow in the style tissue, the quantities of these nutrients in the stigma and style were determined. According to Table 3, LGs exhibited far greater protein, total sugar, and reducing sugar concentrations in their style and stigma tissues than did SRT ones in both genotypes. In both genotypes, K concentration in the style + stigma tissues of LGs was somewhat higher than that in SRTs, although this difference was not significant. Contrary to our expectations, greater amounts of Ca, Mg, and B were measured in SRTs than in LGs in both genotypes, although the Ca concentration in 'TN74128' and the Mg concentration in 'TN74243' were not significantly different between the two kinds of flowers (Table 3).

Rylski *et al.* (1984) and Handique & Sarma (1995) reported small quantities of total sugar and reducing sugar in underdeveloped stigmas of SRTs but larger amounts of protein, polysaccharides, and other nutrients in the stigmas of LGs due to their permeable tissues that make them more favorable to pollen grain absorption and germination. These findings are confirmed by Sękara & Bieniasz (2008), who reported lower sugar content in SRTs. The requirements for pollen germination vary from species to species. In addition to moisture, a carbohydrate source is needed for satisfactory pollen germination and tube growth (Dane *et al.*, 2004). Carbohydrates function both as an energy source and a regulator of osmotic pressure (Fei & Nelson, 2003; Huang *et al.*, 2004). One of the best carbon sources for pollen germination and tube growth is sucrose, followed by glucose, fructose, galactose, and lactose (Patel & Mankad, 2014).

Boron (B) also plays an important role in pollen germination and tube growth (Stephenson *et al.*, 1994). By affecting H+-ATPase activity, B promotes pollen germination (Obermeyer & Blatt, 1995). The pollen tube wall is composed of callose, cellulose, and pectin, of which pectin is the major component (Li *et al.*, 2002). Since B plays a direct role in pectin synthesis, it helps the development of the pollen tube membrane (Wang *et al.*, 2003), increases sugar absorption and metabolism (Vasil, 1964), raises oxygen uptake (O'Kelley, 1957), and enhances the pollen response to Ca (Mascarenhas & Machlis, 1964). However, B can be toxic at a higher concentration or trigger deficiency symptoms (Pérez-Castro *et al.*, 2012). Mondal & Ghanta (2012) reported that a low concentration of boric acid stimulated pollen germination and pollen tube growth in *Solanum macranthum*, but at higher concentrations had inhibitory effects. Moreover, Fang *et al.* (2016) showed that a high concentration of B inhibited pollen germination and tube growth in *Malus domestica*. Our findings confirm these results: the far higher B concentration in SRTs than in LGs observed in the present study might have given rise to its inhibitory effect on pollen germination in SRTs.

The results of many early studies indicated that Ca is necessary for pollen germination and pollen tube growth (Ge *et al.*, 2007; Steinhorst & Kudla, 2013). Stephenson *et al.* (1994) emphasized the great reliance of pollen germination and tube growth on Ca content as it plays different roles in protein phosphorylation and enzyme activity of the pollen tube (Polya *et al.*, 1986), in the rigidity of the pollen tube wall (Kwack, 1967), and in the permeability of the pollen tube membrane (Dickinson, 1967). It also helps the growth of the pollen tube tip by controlling vesicle movements toward the tip (Picton & Steer, 1983). In the present study, SRTs exhibited higher Ca concentration than did LGs. This is in agreement with the findings of Belho (1992), who reported that a low concentration of Ca in the culture medium was effective in stimulating pollen germination and pollen tube growth in *Solanum khasianum* and Solanum marginatum, while a high concentration inhibited pollen germination and reduced pollen tube elongation. K and Mg are also known to stimulate pollen germination and tube growth (Shivanna & Johri, 1985; Taylor & Hepler, 1997). These two ions in the culture medium serve as ions supporting the stimulatory effects of Ca (Brewbaker & Kwack, 1963). K and Mg have been reported to have stimulatory effects on pollen germination and tube growth in *Solanum marginatum*, but inhibitory effects in *Solanum khasianum* (Belho, 1992). Germination and the number of long pollen tubes decreased in the latter species with increasing pollen K and Mg contents (Belho, 1992). In accordance with these results, in the present study the higher amounts of Mg measured in SRTs had inhibitory effects, while K, with nearly the same amounts in LGs and SRTs, did not exhibit any inhibitory effects.

Micromorphological traits

Fig. 1 presents cross sections of LG and SRT styles of 'TN74128'. These data shows that the surface area of the central canal is larger in LG (418 μm^2) than in SRT (283 μm^2). In addition, the number of vascular bundles in the LG style (6) is higher than in SRT (4). These canals are filled with pollen tube transmitting tissue. The nutritional role of this tissue has shown that pollen tubes nourish from products of cells comprising this tissue (Erbar, 2003). On the other hand, since vascular bundles also transport water, nutrient and organic molecules, increasing the number of vascular bundles in LGs can increase the growth of pollen tubes toward the ovules.

Pollen grain transfers male gametes to the female part of the flower playing an important role in the success rate of fruit setting. High crop yield typically depends on viable pollens. Pollen's critical role has motivated further investigation of its features. According to Table 3, LGs in 'TN74243' recorded a greater pollen number than SRTs did, while this difference was not significant in 'TN74128'. The polar axis and equatorial diameters of

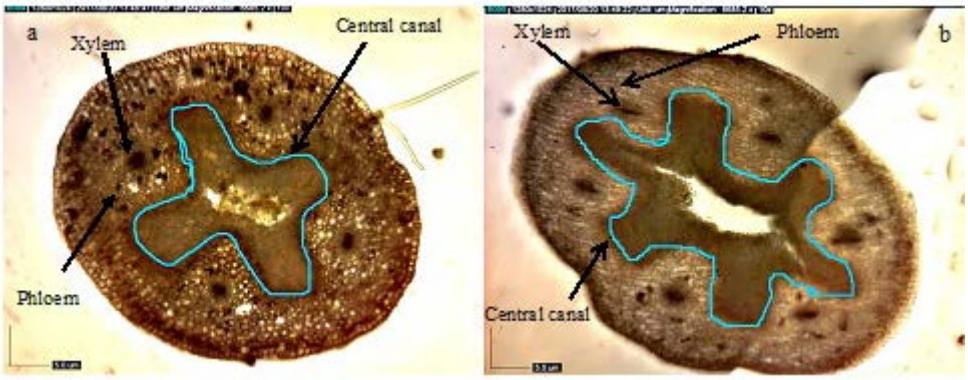

Figure 1. Cross section of style tissue in short style flower (a), and long style flower (b) of eggplant 'TN74128'. Scale bar 5.0 µm

the pollens were measured to determine pollen shapes. In both genotypes, the polar axis of the pollen grains in LG was higher than that of SRT (Table 3). No significant differences were observed, however, in equatorial diameter between the two types of flowers. Fig. 2 presents the polar axis and equatorial diameter of pollen grains in two kinds of flowers in 'TN74128'. Rylski *et al.* (1984) found no differences in pollen number, shape, size, or fertility between these two types of flowers in the studied varieties.

Pollen viability was determined using the two chemicals TTC and IKI. The IKI test failed to distinguish viable from non-viable pollen grains, as they all turned black in this test. TTC, however, showed significant differences between the two types of flowers (Fig. 3a). In both 'TN74128' and 'TN74243', 60.67% and 59% of the LG pollen grains were found to be viable, while only 39.5% and 35% of the SRT ones were identified as viable, respectively (Table 3). The *in vitro* pollen germination method was also conducted to find differences in pollen germination between LGs and SRTs (Fig. 3b). This germination was insignificant after 2, 4, 6, and 8 hr; about 60% of the pollen grains in both LGs and SRTs germinated after 8 hr in both genotypes (data not shown). Pollen tube growth was also examined after 8 hr to find significantly longer tubes in LGs than those in SRTs in both genotypes (Table 3). Simple and fast, this test method (*in vitro* pollen germination) is most commonly used for determining pollen grain viability. The IKI test overestimated pollen viability as it identified all the pollen grains from both LGs and SRTs as viable. Compared with the *in vitro* pollen germination test, the TTC test also underestimated pollen viability in SRTs as it identified as viable only 37% of their pollen grains. Both tests, however, showed LG pollen viability percentages to be identical. Shivanna & Rangaswamy (2012) found that the IKI test would not be useful for assessing pollen viability, since such non-vital stains as iodine in potassium iodide (IKI), acetocarmine, and aniline blue in lactophenol import color to both fresh and dead pollen grains alike. Hence, all the pollen grains in this study turned black after using IKI. As for the

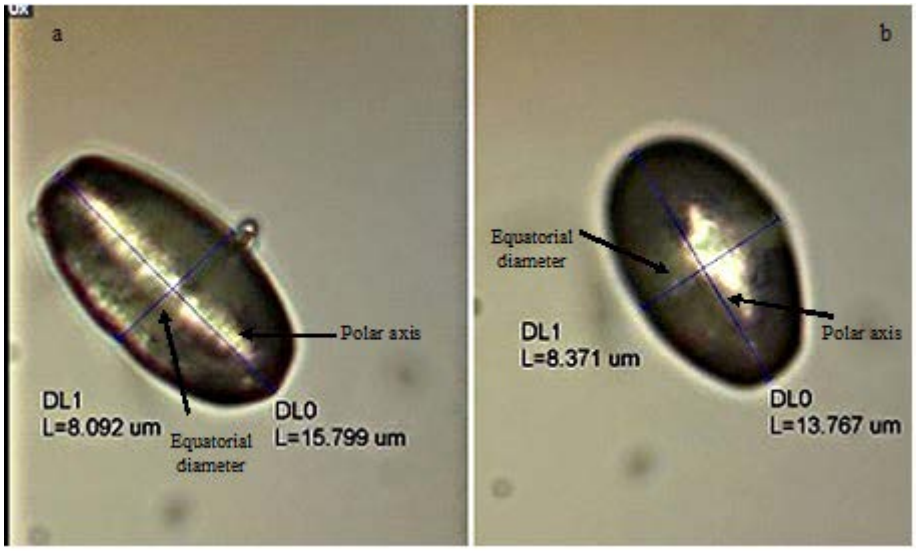

Figure 2. Polar axis and equatorial diameter of the pollen grain in long style flower (a), and short style flower (b) of eggplant 'TN74128'

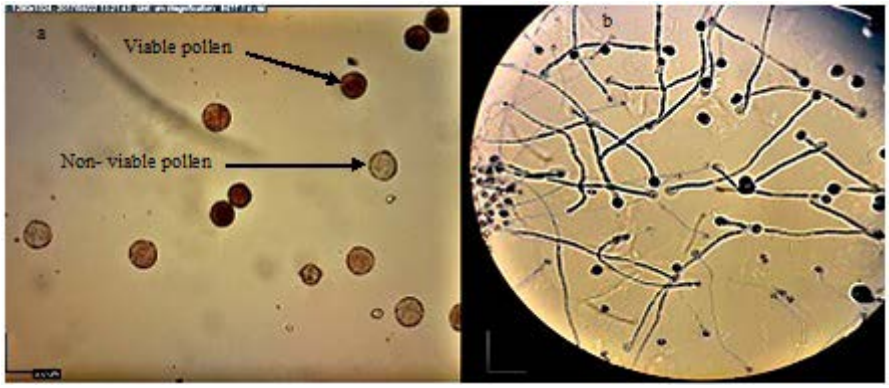

Figure 3. Using triphenyl tetrazolium chloride (TTC) for pollen viability: red color shows viable pollen and the bright one shows non-viable pollen (a), and pollen germination and pollen tube growth from in vitro pollen germination test (b). Scale bar 5.0 µm

tetrazolium test, despite the satisfactory results reported in many species, no positive correlation has been established between the tetrazolium and *in vitro* pollen germination test results in some other species (Dafni *et al.*, 2005). Furthermore, a graduated color (from very bright to deep red) reportedly developed in pollen grains stained in this method, such that setting a boundary point to distinguish between viable and non-viable pollen grains would be quite subjective (Shivanna & Rangaswamy, 2012).

Effect of NAA and Spd on flowering and initial fruit setting

As seen in Table 4, in both genotypes application of 1.0 and 1.5 mM Spd led to significant increases in the percentage of LG+ME in both solitary and inflorescence flowers compared to the control. Although treatment of 20 mg L^{-1} NAA had the same effect on increasing the percent of LG+ME, its difference was not significant compared to the control. Moreover, in 'TN74128' results obtained from higher concentrations of NAA (30 and 40 mg L^{-1}) in both solitary and inflorescence type were very similar to the control and differences were not significant. On the other hand, in 'TN74243', although 30 and 40 mg L^{-1} NAA decreased the percentage of LG + ME and increased the percentage of SRT in both solitary and inflorescence type, however they didn't show a significant difference to the control.

In 'TN74128', although 1.5 mM Spd and 20 mg L^{-1} NAA increased the percentage of initial fruit setting from solitary flowers, they did not show a significant difference

Table 4. Effect of spermidine (Spd) and 1-naphthaleneacetic acid (NAA) on flowering of two eggplant genotypes base on style length in two types of flower (solitary and inflorescence).

	Control	Spd (mM)			NAA (mg L^{-1})		
		0.5	1	1.5	0.5	1	1.5
TN74128 (Solitary)							
LG+ME%	70.28^{c}	75.14^{bc}	83.61^{a}	80.07^{ab}	74.15^{bc}	70.85^{c}	70.63^{c}
SRT%	29.71^{a}	24.86^{ab}	16.38^{c}	19.92^{bc}	25.84^{ab}	29.14^{a}	29.36^{a}
TN74128 (Inflorescence)							
LG+ME%	43.82^{c}	44.68^{c}	55.21^{a}	52.27^{ab}	46.04^{bc}	43.79^{c}	42.29^{c}
SRT%	56.17^{a}	55.31^{a}	44.78^{c}	47.72^{bc}	53.95^{ab}	56.20^{a}	57.70^{a}
TN74243 (Solitary)							
LG+ME%	81.80^{cd}	75.91^{cd}	95.08^{a}	92.90^{ab}	83.66^{bc}	79.02^{cd}	73.39^{d}
SRT%	18.19^{ab}	24.08^{ab}	4.91^{d}	7.10^{cd}	16.33^{bc}	20.97^{ab}	26.60^{a}
TN74243 (Inflorescence)							
LG+ME%	43.82^{c-e}	46.08^{b-d}	49.44^{ab}	53.23^{a}	47.34^{bc}	38.78^{e}	41.72^{de}
SRT%	56.17^{a-c}	53.91^{b-d}	50.56^{de}	46.76^{e}	52.64^{cd}	61.21^{a}	58.27^{ab}

LG: long style flower. ME: medium style flower. SRT: short style flower. In each row, values with at least one same letter do not have a significant difference at the 5% probability level according to the LSD test.

with the control, while these treatments significantly increased the percentage of initial fruit setting from inflorescence flowers. Both of these treatments also increased the percentage of the total initial fruit set, but just 20 mg L^{-1} NAA was significant compared to the control (Table 5). This pattern is also present in 'TN74243' in how both 1.5 mM Spd and 20 mg L^{-1} NAA caused an increase of the percentage of initial fruit set from not only solitary type but also inflorescence type, as well as total initial fruit set. However, only 1.5 mM Spd showed a significant difference when compared with the control (Table 5).

In accordance with our results, Ravestijn (1983) found that growth regulators were able to convert SRTs into LGs and MEs, allowing these flowers to set fruits. This author also reported that the best results were obtained with the application of auxin and GA3 among the various growth regulators used to increase fruit setting rates in eggplant (Ravestijn, 1983). Moniruzzaman *et al.* (2015) also investigated the effects of GA3 and NAA, their results showing that all the tested concentrations of the two growth regulators increased LG and ME percentages in eggplants, with the highest value obtained for NAA applied at a concentration of 40 mg L^{-1}. Moreover, Hoque *et al.* (2018) also showed that using 40 mg L^{-1} NAA increased the percentage of LGs and MEs and fruit setting rate of eggplants. Although no published report was found on the effects of polyamines on heterostylous species, the association of polyamines with flower sexuality or fertility has been reported. Rastogi & Sawhney (1990b) found that the male sterile mutants in tomato plants contained more polyamines than normal plants. Application of putrescine (Put) or Spd at concentrations 1×10^{-3} and 1×10^{-4} mol L^{-1} has also shown significant increases in the number of female flowers in walnut tress (Jizhong *et al.*, 2004).

Effect of NAA and Spd on final fruit set and final yield

Although application of 1.0 and 1.5 mM Spd increased the percentage of LG + ME, and 1.5 mM Spd along with 20 mg L^{-1} NAA generally increased the percentage of the initial fruit set, to our surprise, results revealed that final yields and final fruit setting percentage at none of the Spd and 20 mg L^{-1} NAA concentrations used were significantly different from those recorded by the control treatment in both genotypes (Table 5). This table shows that the percentage of fruit dropping is higher in these treatments compared to the control. This fruit abscission in terms of using NAA may be due to the thinning effect of NAA, or in terms of using both NAA and Spd may be explained by this hypothesis that the plant has a self-regulating mechanism that adjusts excessive plant load due to excessive SRTs by abscising this kind of flower. While the plants that have a high number of LGs turn these kinds of flowers to fruits, they finally adjust their excessive load by abscising some of these fruits before ripening. Our results are consistent with Singh *et al.* (2002) and Sarker *et al.* (2011), who reported that NAA did not affect yield in tomatoes and eggplants, respectively. Khezri *et al.* (2010)

Table 5. Percentage of initial fruit set of two types of eggplant flowers (solitary and inflorescence), final fruit set, fruit dropping and final yield of two eggplant genotypes affected by spermidine (Spd) and 1-naphthaleneacetic acid (NAA).

	Control	Spd (mM)			NAA (mg L^{-1})		
		0.5	1	1.5	0.5	1	1.5
TN74128							
Initial fruit set (solitary)	31.24^{ab}	25.47^{b}	25.92^{b}	33.84^{ab}	38.72^{a}	25.82^{b}	28.63^{ab}
Initial fruit set (inflorescence)	12.40^{c}	12.14^{c}	14.48^{a-c}	16.61^{ab}	17.51^{a}	13.61^{bc}	14.16^{a-c}
Total initial fruit set	18.01^{bc}	16.07^{c}	17.96^{bc}	21.40^{ab}	24.04^{a}	16.92^{c}	17.62^{c}
Final fruit set	9.33^{ab}	10.71^{a}	5.71^{b}	9.48^{ab}	9.05^{ab}	$6.92a^{b}$	7.33^{ab}
Fruit dropping	8.67^{bc}	5.36^{c}	12.25^{ab}	11.91^{ab}	14.98^{a}	10.00^{b}	10.28^{b}
Final yield	1666^{a}	1283^{ab}	1003^{b}	1458^{ab}	1374^{ab}	1155^{ab}	1016^{b}
TN74243							
Initial fruit set (solitary)	16.36^{bc}	$13.16b^{c}$	9.41^{c}	28.94^{a}	23.67^{ab}	19.06^{a-c}	17.69^{a-c}
Initial fruit set (inflorescence)	12.83^{b}	11.66^{b}	13.15^{b}	19.36^{a}	15.40^{ab}	11.86^{b}	11.38^{b}
Total initial fruit set	13.31^{bc}	11.95^{c}	12.50^{bc}	20.80^{a}	17.25^{ab}	13.01^{bc}	12.34^{bc}
Final fruit set	8.43^{ab}	7.39^{ab}	6.74^{b}	9.89^{a}	6.71^{b}	5.55^{b}	6.71^{b}
Fruit dropping	4.88^{ab}	4.55^{b}	5.75^{ab}	10.91^{a}	10.53^{ab}	7.45^{ab}	5.57^{ab}
Final yield	1360^{a}	1184^{ab}	1008^{ab}	1338^{a}	1192^{ab}	922^{b}	1217^{ab}

In each row, values with at least one same letter do not have a significant difference at the 5% probability level according to the LSD test.

also showed that, unlike Put, spermine (Spm) increased pistachio yield, while Spd had no effect on pistachio yield (although it reduced fruit abscission). However, other studies found that foliar spraying of NAA on eggplants increased the fruit number and yield of this crop (Singh, 2010; Moniruzzaman *et al.*, 2015; Hoque *et al.*, 2018). Akhtar *et al.* (1997) also investigated the effects of NAA at concentrations of 25, 50, 75, and 100 mg L^{-1} on tomato plants observing that while the yield reached its highest level at a concentration of 25 mg L^{-1}, it gradually decreased with an increasing NAA concentration. Using Put and Spd sprays on date flower clusters, Tavakoli & Rahemi (2014) found that both polyamines increased yield and reduced fruit abscission relative to the values recorded by their control. Based on the results of this study and others, it may be concluded that the timing of polyamine application and the concentrations used are important factors. Moreover, no definitive mechanism can be claimed for polyamines since different effects have been reported for different types of polyamines on different plant species under different treatment conditions. The effects of polyamines on flower development thus await further research. Although this present study has several before-mentioned strengths, an important limitation that should be addressed is that among all three kinds of polyamines, we studied just Spd that we found more effective on flowering, while in regard to the different effects of three kinds of polyamines, further research should be done with all of them.

In general, smaller stigmas in SRTs lead to less pollen grain absorption. Moreover, the low nutrition (protein, total sugar and reducing sugar) of the stigma surface reduces the possibility for pollen germination. Although these types of flowers showed greater Ca, Mg, and B concentrations, the high concentrations of these nutritional elements may have had inhibitory effects on pollen germination and pollen tube growth. SRTs also showed shorter pollen tubes and lower pollen germination percentages, as vital parameters for fertilization and fruit setting. Thus, the decreased pollen germination percentage and pollen tube growth might underlie the failure of SRTs to set fruits. Further study is required, however, to shed more light on the causes underlying this failure. On the other hand, although 1 and 1.5 mM Spd increased LG and ME percentages, and 1.5 mM Spd along with 20 mg L^{-1} NAA increased the total initial fruit setting, they did not lead to any significant increases in the final yield and final fruit set when compared with the control. A possible explanation for this result might be that the plant has a self-regulating state. This means that when it has more SRTs it adjusts its load by abscising this kind of flower, while when it has less SRTs but more LGs, it adjusts its load by abscising its fruits before turning to the harvestable fruits. Although we could increase the LG to SRT proportion and initial fruit set by using plant growth regulators in this study, an important but unanswered question is how we can protect these initial fruits to increase final yield and final fruit set. Therefore, further work is needed to determine how to prevent fruit abscission.

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