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Style length and flower morphology of three eggplant (*Solanum melongena* L.) cultivars from Iran affected by fruit load

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Abstract: A common feature of eggplant is its heterostyly. Long-style flowers bear fruits whereas short style ones fail to do so. Heterostyly is influenced by some factors such as genotype, climatic conditions and fruit load. In this study three eggplant cultivars from Iran were cultivated under greenhouse condition. The influence of presence of fruit (two fruits and four fruits) or absence of that on style length and some other flower morphological was studied in three positions of single, basal and additional. The presence of fruit, specially four fruits reduced style length, stigma width as well as mass of flower, pistil and stigma compared to the control in all times during fruit growth, and after fruit harvest they increased again. Fruit load didn't affect the number of stamen and stamen length. These effects were observed in all three positons of single, basal and additional flowers of all three cultivars. Generally this study showed that fruit load has decreasing effect on style length and size of flowers forming after fruit setting, which reversed after fruit harvesting.

Key words: eggplant; floral morphology; heterostyly; presence of fruit; style length

Vpliv števila plodov na dolžino vrata pestiča in morfologijo cveta pri treh sortah jajčevca (*Solanum melongena* L.) v Iranu

Izvleček: Splošno poznana lastnost jajčevca je heterostilija. Cvetovi z dolgim vratom cvetiča imajo plodove, tisti s kratkim vratom pa ne. Na heterostilijo vplivajo nekateri dejavniki kot so genotip, podnebne razmere in obloženost s plodovi. V raziskavi so bile gojene tri sorte jajčevca v rastlinjaku. Preučevan je bil vpliv števila plodov (dva in štirje plodovi) in njihova odsotnost na dolžino vrata pestiča in nekatere druge morfološke lastnosti cvetov v odvisnosti od njihovega položaja in sicer posamezni, prvi v socvetju in naslednji. Prisotnost plodov, še posebej štirih, je zmanjšala dolžino vrata pestiča, širino brazde kot tudi maso cveta, vratu in brazde pestiča v primerjavi s kontrolo v celotnem obdobju rasti. Po obiranju plodov se je vrednost teh parametrov spet povečala. Obloženost s plodovi ni vplivala na število prašnikov in njihovo dolžino. Ti učinki so bili opaženi pri vseh treh položajih plodov (posamezni, prvi in naslednji), pri vseh treh sortah. Na splošno je raziskava pokazala, da je obloženost s plodovi vplivala na zmanjšanje dolžine vratov pestiča in velikosti cvetov, ki so nastali po zasnovi plodov, po njihovem obiranju pa so se vrednosti teh parametrov spet povečale.

Ključne besede: jajčevec; morfologija cvetov; heterostilija; prisotnost plodov; dolžina vratu pestiča

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1 INTRODUCTION

Eggplant (*Solanum melongena* L.) from the Solanaceae family, is belonging to tropical and subtropical regions (San José et al., 2016). India is primary centre of origin (Meyer et al., 2012); China and Japan are secondary centres of origin, and today this crop is cultivated worldwide from Mediterranean to Africa, Europe and America (Frary et al., 2007; Daunay, 2008). Its total world annual production reached over 55 million tons in 2019, which with an annual production of 670158 tons, Iran is the fifth leading eggplant producer after China, India, Egypt and Turkey (Faostat, 2019).

Flowering and fruit setting are the most important factors in determining the yield of eggplant that is influenced by the genotype, the environmental conditions, the flower's position on the plant and fruit load (Mohideen et al., 1977; Nothmann et al., 1983; Sun et al., 1990; Kowalska, 2003, 2006; Banik et al., 2018). Eggplant flowers are large and usually violet-colored. They consist of five united and persistent sepals, five united and cup-shaped petals, usually five stamens alternating with the corolla, united carpels, and superior ovaries arranged either singly or in inflorescence. The number of flower bud is different in inflorescences and it is between 2-7 flower buds (Rashid & Singh, 2000; Hazra et al., 2003; Jagatheeswari, 2014). Eggplant flowers reportedly exhibit a form of heterostyly. Based on this property, eggplant flowers are classified into the three groups of long style, medium style, and short style flowers based on the style length relative to that of the stamen (Prakash, 1968; Rylski et al., 1984; Handique & Sarma, 1995; Prasad & Sękara & Bieniasz, 2008). Style length plays an important role in the fruit set of eggplant since pollen liberated from pores at the apex of the anther cone thereby promoting the fertilization of long-styled pistils (Rylski et al., 1984; Passam & Bolmatis, 1997). Though short style flowers are not totally infertile, their fruit setting rate is much less than those of long and medium style flowers (Srinivas et al., 2016; Pohl et al., 2019). Since a considerable portion of eggplant flowers are short style ones, their failure to set fruit decreases their fruit-yielding potential significantly (Chadha & Saimbhi, 1977). Although heterostyly in eggplant flowers is a varietal characteristic (Rylski et al., 1984; Kowalska, 2006; Sękara & Bieniasz, 2008), it is affected by such factors as plant age, fruiting dynamics, and environmental conditions (Lenz, 1970; Sun et al., 1990). For instance, Nothmann et al. (1983) showed that low temperatures may have an adverse effect on fruit setting by reducing the length of style. Some researchers have also shown that treatment of seeds with gamma rays caused a change in heterostyly by increasing the long style flowers and decreasing the short style ones (Handique & Sarma, 1995). Moreover, exogenous application of some plant growth regulators such as auxin and kinetin was effective on changing heterostyly by changing the proportion of long and short style flowers (Lenz, 1970; Handique & Sarma, 1995; Passam et al., 2001). Moniruzzaman et al. (2015) and Hoque et al. (2018) also reported that using IAA, significantly increased the percentages of long and medium style flowers in eggplant.

Although the effect of fruit load on style length of some cultivars has previously been studied by some researchers (Khah et al., 2000; Passam et al., 2001), in the present paper we studied this effect on style length and some other flower morphological traits in all three positions of single, basal and additional flowers of three eggplant cultivars from Iran.

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS

Three eggplant cultivars (TN74128, TN74243 and TN74239) obtained from the Gene Bank of the Agricultural Research Institute of Iran were used in this study. From each cultivar, 60 seeds were sown in the middle of March into boxes with a peat and perlite substrate at a ratio of 4:1; v/v. Six weeks after sowing, 18 uniform seedlings were selected from each genotype, and transplanted into an experimental field at spaces of 60 × 60 cm at Isfahan University of Technology, Isfahan, Iran (latitude 32° 42′ N; longitude 51° 28′ E; altitude 1624 m). The soil of the experimental site was sandy loam with neutral pH suitable for cultivation. Field was ploughed 2-3 times and organic manure 25 t ha^{-1} was applied. The fertilizer 20-20-20 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}. Plants were irrigated using the drip irrigation method, and weeding of the field was carried out several times manually. Pest management were conducted according to recommended standards during the growing season.

2.2 TREATMENTS AND OBSERVATIONS

In this trial three treatments applied including: 1) all flowers collected at anthesis (NF), 2) two fruits allowed setting, but all subsequent flowers collected at anthesis (2F), 3) four fruits allowed setting and all subsequent flowers collected at anthesis (4F). Each treatment comprises 9 plants that all of them were samples through the trial. In all three cultivars, single flower that were formed in the third week were kept on plants for turning into fruit (for treatments of 2 fruits and 4 fruits), and from the fifth week, studies relevant to the flowers forming after fruit setting were carried out. For this purpose, flowers were excised twice a week. They were classified according to whether they were single, basal (first flower in inflorescence) or additional (second flower in inflorescence) flower. Then they immediately transferred to the laboratory. It was assessed the number of stamen, the length of style and stamen, the width of stigma, and the mass of flower, pistil and stigma. The length of style and stamen, as well as the width of stigma were measured using a caliper on the millimeter scale (style and stamen length measurements were made from the point of attachment of the style to the ovary). Flower, pistil, and stigma mass were also measured using a sensitive digital scale and reported in milligram (flower and pistil), and microgram (stigma). The ninth week, fruits were harvested, and the observations lasted two weeks later, until the eleventh week.

2.3 DATA ANALYSIS

The results were statistically analysed by analysis of variance (ANOVA) using the SAS software, and in order to compare differences, least significant differences (LSD) test ($p < 0.05$) was applied for traits with significant F in ANOVA. Due to the lack of coordination of different treatments at the time of measuring the factors, the resulting data obtained from the first four weeks of the experiment (before fruit setting) was carried out with Split-plot according to randomized complete block design, included the position of flowers on the plant (single, basal or additional). For plants without any fruits, and in order to analyse the data from the fifth to eleventh week was used the split-plot factorial based on randomized complete block design. Treatments included the number of fruits per plant (0, 2 and 4 fruits) and flower position on the plant (single, basal or additional) in 3 replications and each replicate contains 3 plants.

3 RESULTS

3.1 STYLE LENGTH AND OTHER FLOWER MORPHOLOGICAL TRAITS AFFECTED BY FRUIT LOAD

As can be seen in Table 1, the presence of two and especially four fruits reduced the style length of the flowers that were later formed. The presence of fruit also significantly affected the size of the other female parts including width of stigma and mass of pistil and stigma, which reduced them in all three cultivars. The presence of four fruits per plant reduced the mass of the next flowers significantly in all three cultivars. The number and the length of stamen in any of the cultivars were not influenced by the number of fruits (data not shown).

3.2 STYLE LENGTH AND OTHER FLOWER MORPHOLOGICAL TRAITS BASED ON FLOWER POSITION

As expected, in Table 2, all traits measured included style length, number of stamens, stamen length, stigma width and the mass of flower, pistil and stigma were significantly less in additional flowers in all three cultivars compared to the basal and single flowers. Some of these traits are not significant between the basal and single flowers, and some of them in the basal flowers are a little more than single ones.

3.3 FLOWER MORPHOLOGICAL TRAITS AT DIFFERENT STAGES OF FRUIT GROWTH AFFECTED BY FRUIT LOAD

3.3.1 Style lenght

As shown in Fig. 1, in cultivar TN74128, the minimum and maximum (min and max) of style length in the basal flower were 13.41 and 14.31 mm, up to the 9th week in NF, while were 12.88 and 13.45 mm in 2F, and 12.03 and 12.33 mm in 4F, which increased to 13.30 mm in the 10th and 11th weeks after harvesting of fruits in 4F. This factor in the additional flower was 8.35 and 12.56 mm in control, 5.73 and 9.93 mm in 2F, and 4.1 and 9.36 mm in 4F, which increased after fruit harvest compared to the 9th week. In single flowers, this value was 13.06 and 13.58 mm in control, which did not differ significantly with 2F. While in 4F were 11.31 and 12.10 mm, which increased to 13.01 mm after harvest.

The min and max style length of basal flower in cultivar TN74243 were 11.40 and 12.59 mm until the 11th week. There was no significant difference in 2F with control. But in 4F it was 10.65 and 11.68 mm, which increased to 12.27 mm after the 9th week. In the additional flower, a significant decrease in the style length of all three treatments was observed from the 5th week to the 7th that began to increase after the 9th week. For single flower, this was 11.71 and 13.07 mm in control. In

Table 1: Effect of fruit load on style length and some flower morphological traits in three eggplant cultivars

Fruit load	Style length (mm)	Stigma width (mm)	Flower mass (mg)	Pistil mass (mg)	Stigma mass (µg)
Cultivar TN74128					
No fruit	12.72a	1.84a	0.88a	0.17a	2.70a
2 fruit	11.53b	1.67b	0.87a	0.16a	2.15b
4 fruit	10.34c	1.56c	0.73b	0.13b	1.80c
Cultivar TN74243					
No fruit	10.66a	1.74a	0.86a	0.15a	2.36a
2 fruit	9.88b	1.65ab	0.83a	0.14b	1.97b
4 fruit	8.94c	1.58b	0.78b	0.11c	1.80c
Cultivar TN74239					
No fruit	9.76a	1.52a	0.76a	0.12a	2.0a
2 fruit	9.20b	1.45b	0.71b	0.11b	1.67b
4 fruit	8.41c	1.35c	0.66c	0.10c	1.48c

The values with similar letters in each column for each cultivar have no significant difference using LSD test at 5 % probability.

Table 2: Differences between position of flower in the style length and other flower morphological traits of three eggplant cultivars

Flower position	Style length (mm)	Number of stamen	Stamen length (mm)	Stigma width (mm)	Flower mass (mg)	Pistil mass (mg)	Stigma mass (µg)
Cultivar TN74128							
Basal	13.29a	6.67a	14.14a	1.98a	1.03a	0.21a	3.08a
Additional	8.38c	6.33c	13.78b	1.21b	0.46b	0.04b	0.71c
Single flower	12.92b	6.55b	14.04a	1.89a	0.99a	0.21a	2.86b
Cultivar TN74243							
Basal	11.93a	6.32a	14.24a	1.89a	1.01a	0.19a	2.98a
Additional	5.63b	6.10b	13.81c	1.14b	0.46b	0.032b	0.35c
Single flower	11.92a	6.34a	14.00b	1.94a	1.01a	0.18a	2.80b
Cultivar TN74239							
Basal	11.51a	6.40a	14.18a	1.66a	0.88a	0.16a	2.53a
Additional	4.30b	5.92b	13.68b	1.08c	0.38b	0.02c	0.25c
Single flower	11.56a	6.33a	14.09a	1.58b	0.86a	0.15b	2.36b

The values with similar letters in each column for each cultivar have no significant difference using LSD test at 5 % probability

2F were 11.21 and 12.25 mm, which increased to 12.46 mm after harvest, and in 4F were 10.78 and 11.93 mm, which no difference was created after the 9th week.

The min and max style length of the basal flower in cultivar TN74239 was 11.19 and 11.84 mm until the 11th week. 2F did not show any significant difference with control, while in 4F, were 10.71 and 11.01 mm, which increased to 11.6 mm in the 11th week. In the additional flower, from the 5th week to the 8th and 9th weeks, there was a significant decrease in the style length of all three treatments that increased from the 10th to 11th weeks. The min and max style length of single flower was 11.10 and 12.49 mm in NF, and no significant difference was observed between 2F with control, while this amount in 4F was 10.15 and 11.0 mm, which increased to 12.45 mm after harvest.

3.3.2 Stigma width

According to Fig. 2 in cultivar TN74128, the min and max width of stigma in basal flower in control was 1.97 and 2.31 mm up to the 9th week, 1.75 and 2.35 mm in 2F, and 1.49 and 2.18 mm in 4F, which increased in two last treatments in the 10th week compared to the 9th week. There was also the similar trend in the additional flowers. In single flower, this factor was 1.95 and 2.14 mm in control, 1.71 and 2.02 mm in 2F, and 1.55 and 2.07 mm in 4F, which increased at the 10th and 11th weeks in both last treatments.

There was no significant difference between control and 2F in stigma width of basal flower in cultivar TN74243, while in 4F at all times, especially on the 9th week, the width of the stigma was less than the control and 2F, which increased after fruit harvest. The stigma width of the additional flowers had a sharp decrease in all three treatments from the 5th week to the 8th and 9th weeks, and then increased at the 10th and 11th weeks. In single flower, these were 1.89 and 2.26 mm in control, while was lower in 2F and 4F, which increased at the 10th and 11th weeks.

In cultivar TN74239, at all times, the stigma width of the basal flower was lower in 2F and 4F with a slight difference compared to the control, and after harvest increased. The additional flower showed a decrease trend from 5th week to 8th week in all three treatments and then began to increase. Min and max of stigma width in single flower were 1.52 and 1.68 mm in control until the 9th week. There was no significant difference in 2F, and in 4F were 1.18 and 1.7 mm, which increased after fruit harvest in both treatments.

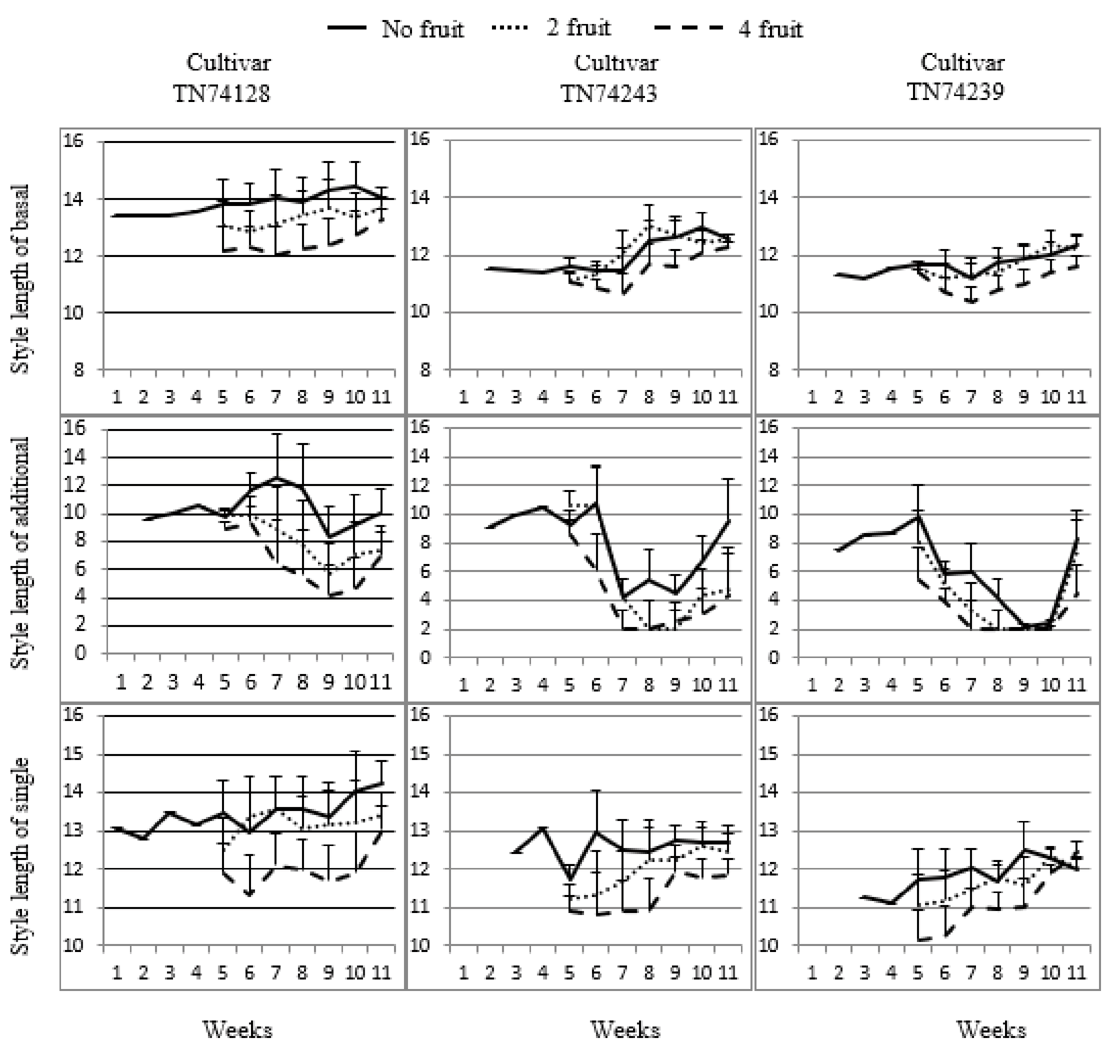

Figure 1: Style length of basal, additional and single flower in millimeter in three eggplant cultivars along the growing period affected by fruit load. Vertical bars represent standard deviation of the means

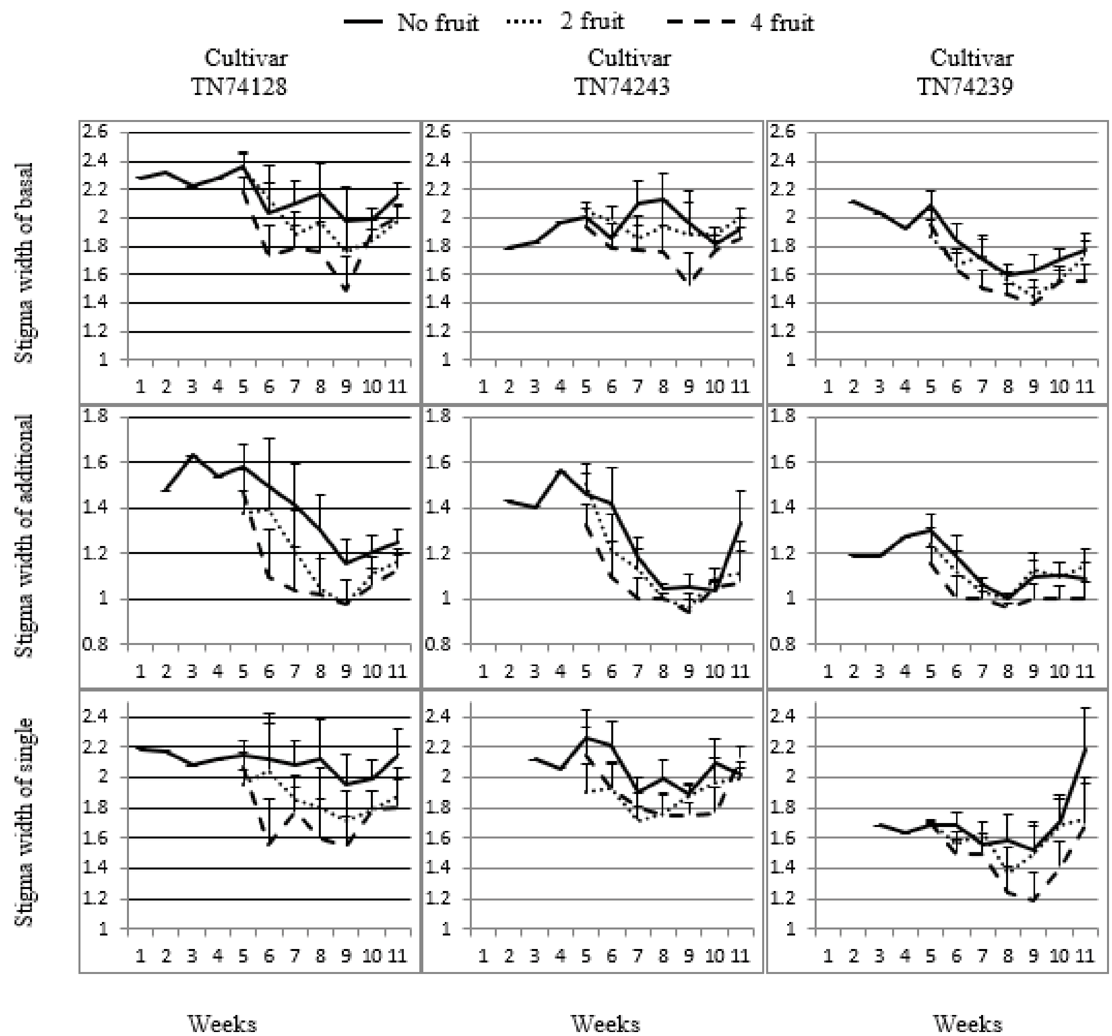

Figure 2: Stigma width of basal, additional and single flower in millimeter in three cultivars of eggplant along the growing period affected by fruit load. Vertical bars represent standard deviation of the means

3.3.3 Flower mass

No significant differences were found between 2F and 4F with control in basal and additional flowers of all cultivars. But in single flower the min and max flower mass of control in cultivars TN74128, TN74243 and TN74239 was 1.03 and 1.46, 0.96 and 1.33, and 0.88 and 1.20 mg, respectively, while in 4F, this factor was significantly lower (0.74 and 1.09, 0.80 and 1.20, and 0.71 and 0.89 mg, respectively), which increased slightly after harvest (Fig. 3).

3.3.4 Pistil mass

The min andmax pistil mass of basal flower in cultivar TN74128 until the 9th week was 0.16 and 0.33 mg in control. 2F did not show any significant difference with control, while in 4F was 0.13 and 0.22 mg, which showed a significant increase after harvesting compared to the last weeks. This factor in additional flower was 0.027 and 0.074 mg in control, 0.026 and 0.058 mg in 2F, and 0.012 and 0.044 mg in 4F, which after the harvest increased. In single flower, this amount was 0.22 and 0.34 in control, 0.15 and 0.28 in 2F and 0.15 and 0.2 mg in 4F, which after harvest increased compared to the 9th week.

The min and max pistil mass of basal flower in cultivar TN74243 in control was 0.15 and 0.26 mg. 2F didn't have significant difference with control, and in 4F, it was 0.14 and 0.22 mg. The pistil mass of additional flower was significantly reduced in all three treatments from the 5th week to the 8th week, and after the 10th week it increased slightly. In single flower, there was no significant difference between 2F with control, but in 4F was lower than the control at all times, which increased after the 9th week.

In cultivar TN74239, the pistil mass of basal flower was not significantly different in 2F and 4F with control, although this factor was less than the control at all times. In the additional flower, the pistil mass decreased significantly from the 5th to 7th weeks in all three treatments and increased slightly after the 10th week. In the single flower, 2F did not show any difference with the control, but in 4F at all times, the mass of the pistil was less than that of the control and after harvest increased compared to the previous one (Fig. 4).

3.3.5 Stigma mass

In cultivar TN74128, the min and max stigma mass of basal flower until the 9th week in control was 3.16 and 3.91 µg, in 2F, 2.53 and 3.73 µg, and in 4F, 1.99 and 3.04 µg, which increased after fruit harvest. Min and max of this factor in additional flower of control, 2F and 4F was 0.78 and 1.28, 0.58 and 0.75, and 0.37 and 0.46 µg, respectively, which increased slightly after the 9th week. In single flower, this factor was 2.22 and 4.22 µg in control, 2.36 and 2.85 µg in 2F, and 1.73 and 2.67 µg in 4F, which at the 10th and 11th weeks was more than to the previous weeks.

In cultivar TN74243, the min and max stigma mass of basal flower in control was 2.44 and 3.66 µg. This amount in 2F was 2.20 and 3.05 µg and in 4F was 2.10 and 2.75 µg, which increased in all three treatments after the 9th week. The stigma mass of the additional flower was 0.25 and 0.62 µg in the control. In 2F, 0.10 and 0.35 µg, and in 4F, without significant dif-

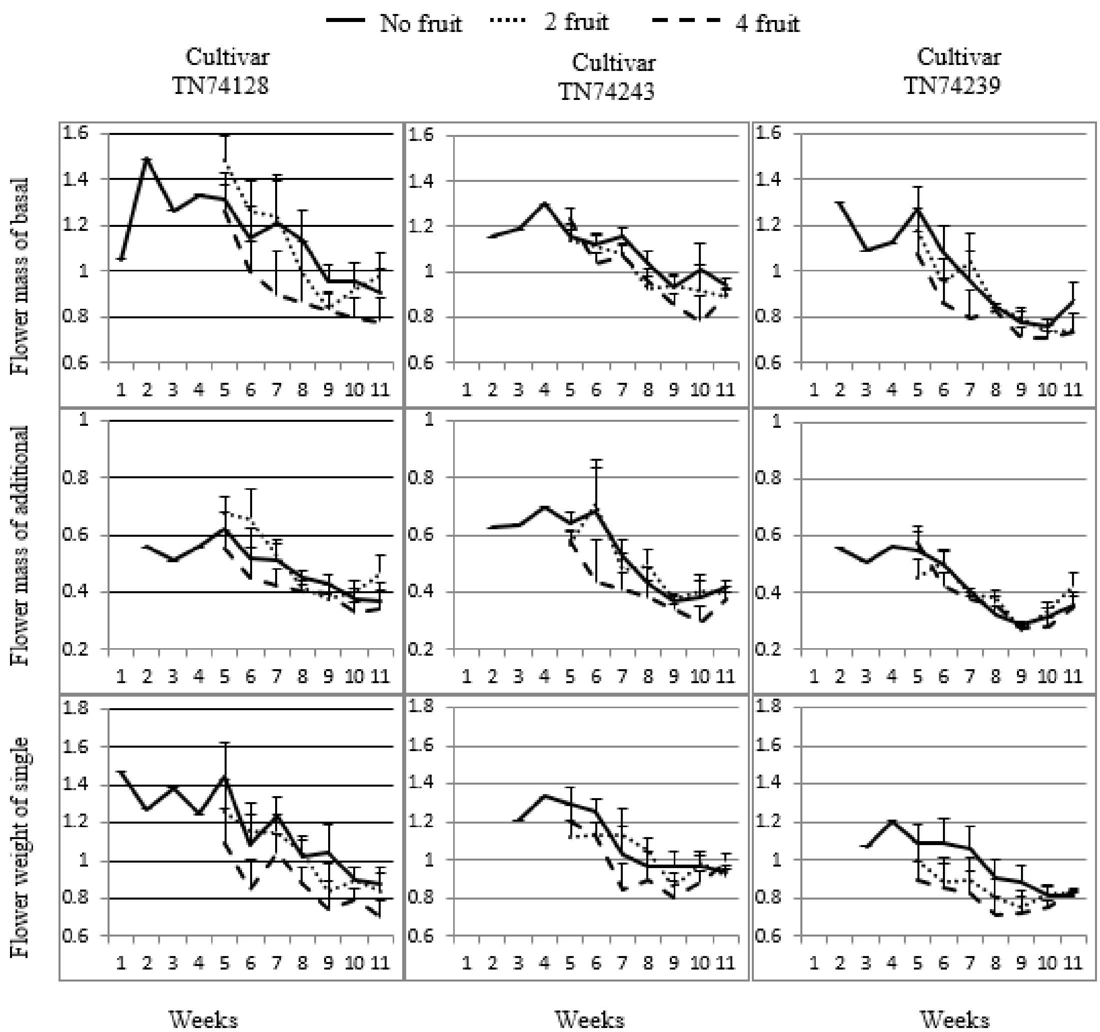

Figure 3: Flower mass of basal, additional and single flower in milligram in three cultivars of eggplant along the growing period affected by fruit load. Vertical bars represent standard deviation of the means

ferences with 2F was 0.10 and 0.32 µg, which increased after harvest. In single flower, this factor was 2.20 and 3.45 µg in control, in 2F, 2.06 and 2.90 µg, and in 4F, 1.66 and 2.85 µg that at the 9th and 10th week reached to their maximum.

In cultivar TN74239, the min and max stigma mass of basal flower in control was 2.12 and 3.57 µg, in 2F, 1.80 and 2.94 µg, and in 4F, 1.98 and 2.52 µg that increased after harvest than previous weeks. This factor showed a significant reduction in additional flowers in all three treatments from 5th to 8th and 9th weeks, while the graph of 2F and 4F were lower than the control, and then increased at the 11th week. The min and max stigma mass of single flower in control was 1.98 and 2.60 µg. This amount in 2F was 1.72 and 2.55 µg, and in 4F, 1.15 and 2.20 µg, which reached to the maximum value in all three treatments in the 10th and 11th weeks (Fig. 5).

4 DISCUSSION

The presence of two or four fruits in all three cultivars reduced the length of style, as well as the width of stigma in all three positions of the next basal, additional and single flowers. This difference was especially significant and remarkable in 4F. This process continued from the beginning until the time of fruit harvesting, and after harvesting began to increase again. The mass of basal and additional flower in three cultivars was not significantly affected by 2F and 4F, while mass of single flower was especially decreased in 4F as compared to the control, which increased again after harvesting. This factor generally decreased from the beginning of flowering to the end of the fruit growth period in all three cultivars. The mass of pistil also showed a similar trend to the mass of the flower during the growth

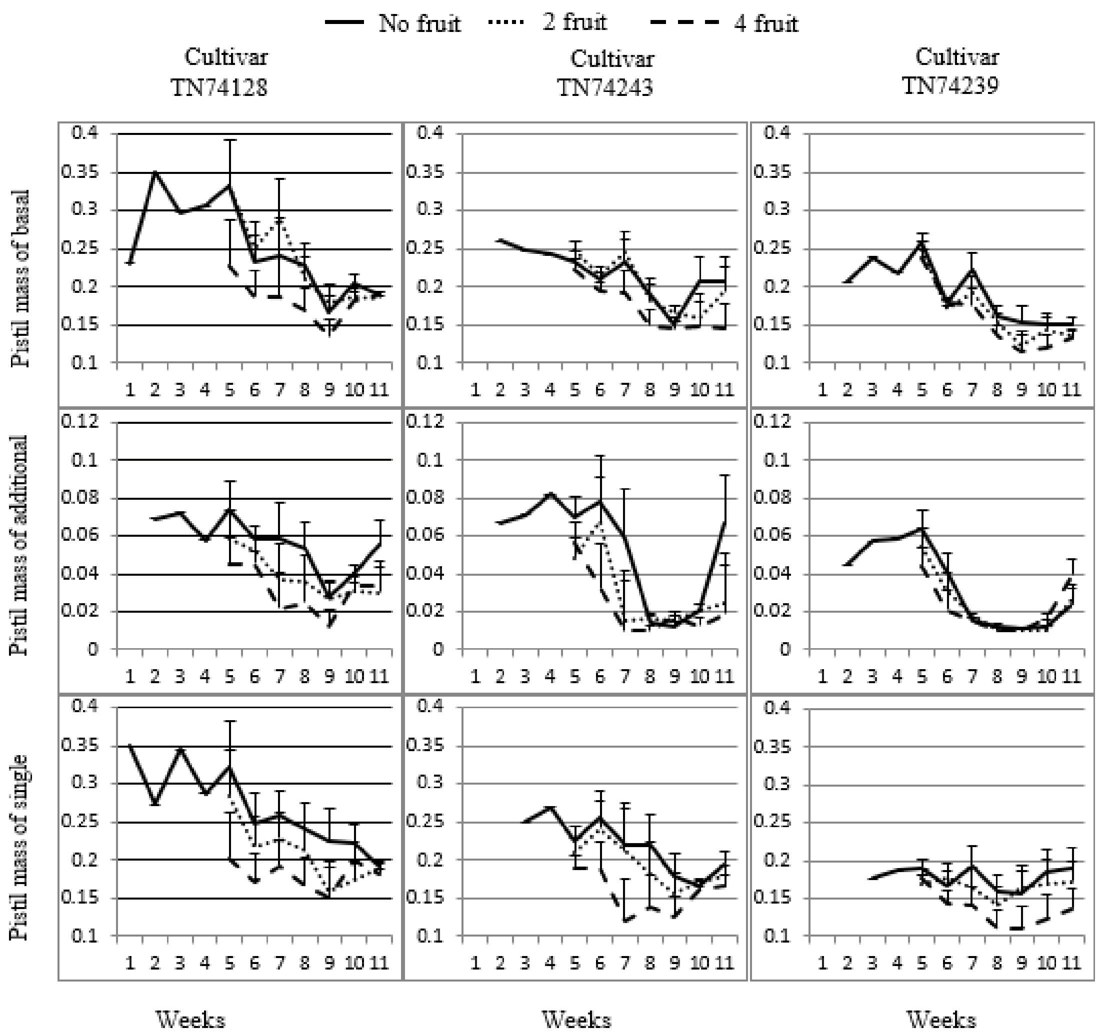

Figure 4: Pistil mass of basal, additional and single flower in milligram in three cultivars of eggplant along the growing period affected by fruit load. Vertical bars represent standard deviation of the means

period. In most cases, 2F did not affect the mass of pistil so much, while in 4F, this factor was more affected, so that it decreased until the fruit harvesting, and after that increased again. The mass of stigma was influenced by two and four fruits during the growth period, so that the presence of fruit per plant in all three cultivars reduced the mass of stigmata of flowers that formed after fruit setting, and after fruit harvest increased again. The treatment of two fruits and four fruits did not affect the number and the length of stamen in any of the cultivars during the growth period.

These results are in agreement with the study of Khah et al. (2000) on four eggplant varieties in the greenhouse conditions. They showed not only the length of style, but also the mass of flower, the mass of pistil and the number of flowers decreased by the number of fruits, and increased again after fruit harvest. Lenz (1970) also examined this issue in Hoagland's culture and expressed that the formation of the fruit affected style length of the next flowers. Moreover, Passam et al. (2001) reported similar results. Their experiments were conducted under favorable climatic conditions for fruit formation. According to them, the presence of fruit caused length of style was smaller during the period of fruit growth, which increased after the maturity of the fruit. The mass of the flower was also affected by the presence of fruits, and this factor also similar to our results showed a downward trend in the trial period. Pistil mass also had a pattern similar to flower mass, and no significant difference was observed in cultivars in stamen length between treatments.

Developing fruits reduce the growth of roots, stems and leaves in eggplants (Mochizuki, 1959), as occurs in other plant species (Leonard, 1962). Lenz (1970)

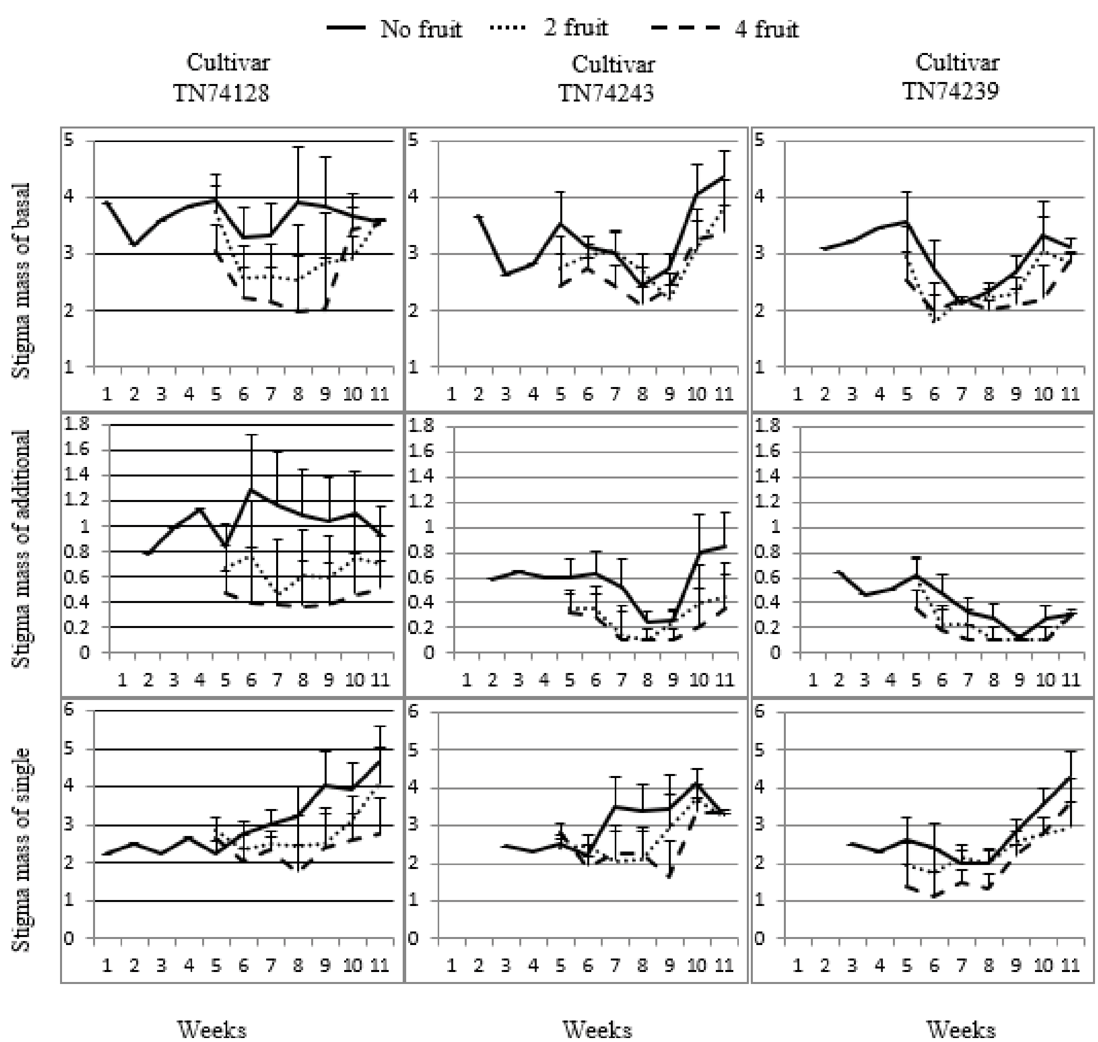

Figure 5: Stigma mass of basal, additional and single flower in microgram in three eggplant cultivars along the growing period affected by fruit load. Vertical bars represent standard deviation of the means

also showed this growth inhibition for flower style. This inhibitory growth is due to the competition of nutrients and assimilates, and may also result in hormones produced in the fruit. According to the Lenz (1970), both the length of style and stamen are influenced by auxin. Therefore, auxin existing in developing fruits can inhibit the growth of the style, which suggests that developing fruits control the sex expression of the eggplant. Claussen (1986) also states that since fruits are strong sink, the use of leaf carbohydrates reduces vegetative growth and decreases flower size. The issue of the dominance of the first fruit in plants from Cucurbitaceae family has also been proven. In these plants, the developing fruit can prevent the formation of the next fruit and the growth of new and young fruits. This inhibition can be due to competition for available assimilates, or due to the dominance of growth regulators created by the developing fruit. The pollen-dependent seed content seems to play a role in the dominance of the first fruit (Stephenson et al., 1988; Bangerth, 1989). However, Passam et al. (2001) demonstrated in a trial that the use of auxin in the absence of fruit did not affect the length of the style. Therefore, they stated that although the seeds of developing fruits through the synthesis of natural auxins or other growth regulators may have some effect on the length of the style, it seems that the main factor is the presence of fruit and the stage of fruit growth.

5 CONCLUSION

In summary, although fruit load did not affect the number and length of stamen of flowers which form later, reduced style length, stigma width, mass of flower, pistil and stigma of those flowers, which increase again after fruit harvesting. Therefore, the presence of fruit on plant reduces the chance of fruit formation from subsequent flowers that shows timely harvest of fruit can be effective in the formation of more long style flowers during plant growth period and so, more fruit setting.

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