Phytohormones Content in Cucumber Leaves by Using Pruning as a Mechanical Stress

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Abstract: The effect of mechanical stress created by pruning of lateral branches on sex expression in cucumber cultivar, “Sialkot Selection” was examined. Seeds were sown in the field and after reaching the required number of lateral branches according to respective treatment, each new coming lateral branch were pinched off. The pruning treatments included; main stem without any lateral branches (T₀) and main stem with one (T₁), two (T₂), three (T₃), four (T₄) and five (T₅) lateral branches. The un-pruned plants were kept as control (T₀). The experiment was conducted under RCBD with three replications. The greatest increase in female flower production, lowest female to male sex ratio, highest number of fruits plant⁻¹ and yield were recorded with the treatment T₁ compared with control. This is due to decrease in endogenous GA level at blooming and fruiting stages and increase in IAA level at flower initiation, blooming and fruiting stages. Yield increase in relation to control recorded up to 61%.

Key words: Pruning · Mechanical stress · Phytohormone level · Sex expression

INTRODUCTION

Mechanical stress is a tool which had been used in the past in different crops for different physiological responses. In baby carrots centrifugation time was used as mechanical stress and in response changes in ethylene evolution and whiteness index were measured [1]. A significant correlation was found between the agitation speed and the morphological changes occurred in the fungal hyphae during the production of tannase in a submerged fermentation system [2]. In another study effects of sinusoidal vibration (40-120 Hz, amplitude equal to or smaller than 0.42 mm) on seed germination of Arabidopsis thaliana were examined and reported that the increase in the rate of germination appeared dependent on acceleration calculated from the frequency and amplitude of vibration [3]. Significant morphological and anatomical changes in the root apical meristem organization of Zea mays were observed by mechanical stress [4]. In tomato fruits harvested at different ripening stages, an increase in respiration and ethylene production was observed after the application of mechanical stress created by double impacts over the locule with a force equivalent to two 40-cm drops, followed by ripening at 20°C [5]. Pruning or maintaining a certain number of branches on a plant is also a mechanical stress. Mechanically-induced stress (MIS) occurs in plants naturally by the movement of aerial parts. It can also be induced by various actions such as rubbing or bending the stem or shaking or brushing the entire shoot [6]. Mechanical stress can also be produced by root pruning [7]. In rice seedlings growth it was observed that greater root dry weight was associated only in root-pruning treatments [8]. In guava yield can be improved by pruning treatments along with spraying chemicals substances [9]. Different species respond differently to these stresses and this question is answerable that what the endogenous control mechanism for MIS responses is. Ethylene, which increases as a result of MIS in several species, may cause some MIS responses such as change in sex expression [10]. Pruning the lateral branches may change the endogenous level of phytohormones and possibly change the partition of assimilates as ethylene released from different organs of the stressed plants with
During the present investigation it was aimed to examine the effects of pruning on sex expression in cucumber (cv. Sialkot Selection) in order to understand the sex ratio and effect on yield as affected by the different treatments of pruning and also endogenous control mechanism of sex expression for some of treatments. The cultivar under study is a monoecious cultivar which has a strong tendency towards maleness which limits yield.

**MATERIALS AND METHODS**

Seeds of cucumber cultivar ‘Sialkot Selection’ were sown in the month of March for two years. The studies were conducted at Vegetable Crops Research Programme, National Agricultural Research Centre Islamabad, Pakistan. Plants were spaced at 2.25 x 0.6 m. Standard production practices for open field cucumbers were followed. Seedlings were protected from the attack of red pumpkin beetle by the dusting of carboxyl and ash in the ratio of (1:10) at 2-4 leaf stage of plant growth.

The pruning treatments included; main stem without any lateral branches (T1) and main stem with one (T2), two (T3), three (T4), four (T5) and five (T6) lateral branches. The un-pruned plants were kept as control (T0). Only number of lateral branches mentioned in the respective treatments was maintained along with the main stem. All other lateral branches arising on the stem during growing period were pinched off regularly. A randomized complete block design (RCBD) with three replications was used. Five plants were included in each replication.

Each plant was observed for 1st staminate & pistillate flower appearance, number of staminate and pistillate flowers, number and weight of fruits plant−1 and average fruit weight. The sex ratio of flowers was calculated on the basis of staminate and pistillate flowers. The data from each experiment were analyzed using the ANOVA procedures for a randomized complete block design with the treatments randomly assigned in three replications. The graphs were plotted using Microsoft Excel.

For the extraction and detection of endogenous hormones (IAA and GA3) contents of plant leaves, collected leaves at four growth stages (two leaves, flower initiation, blooming and fruiting). The extraction and purification was made following the method of Kettner & Drofling [12]. The analysis of IAA and GA3 were made following the methods of Sarwar et al., [13] and Li et al., [14].

The leaves (1g) were ground in (80 %) methanol at 4°C with an antioxidant, butylated hydroxyl toluene (BHT). The leaves were extracted at 4°C in dark for 72 hours with subsequent changes of solvent. The extracted sample was centrifuged and the supernatant was reduced to aqueous phase using rotary thin film evaporator (RFE). The pH of the aqueous phase was adjusted between 2.5 and 3.0 and partitioned four times with ½ volume of ethyl acetate. The ethyl acetate was dried down completely using rotary thin film evaporator (RFE). The dried sample was re-dissolved in 150 µL of methanol (100%) and was analyzed on HPLC (Agilent 1100) using UV detector and C-18 column (39 x 300 mm). For identification of hormones 10 µL sample filtered through 0.45 Millipore filter was injected into column. Pure IAA and GA3 (Sigma, USA) were used as standard for identification and quantification of plant hormones. These growth hormones were identified on the basis of retention time and peak area of standards. Methanol, acetic acid and water (30:1:70) were used as mobile phase. The flow rate was adjusted at 0.5 ml/min with an average time for 20 min/sample. The wave length used for the detection of IAA was 280 nm whereas for GA3 analysis it was set at 254 nm.

**RESULTS AND DISCUSSION**

Days to 1st Staminate and Pistillate Flower: Pruning treatments with 1-5 lateral branches (T1 i.e. main stem with 1 lateral branch to T6 i.e main stem with 5 lateral branches) decreased the time for the appearance of staminate flowers as compared to control (Fig. 1). This could be due to higher level of endogenous GA at flower initiation stage (Table 1) as compared to control. However, the treatments with 3-5 lateral branches i.e. having main stem with 3 lateral branches and main stem with 5 lateral branches decreased the time for the appearance of pistillate flowers. This observed earliness could be due to higher level of endogenous IAA at flower initiation stage (Fig. 2 & Table 1) as compared to control. As role of ethylene and auxin in regulating the growth and morphology of roots during mechanical impedance by using the model plant Arabidopsis (Arabidopsis thaliana) was already investigated [15]. Similar findings were also reported by Erner and Jaffe [16] that mechanical perturbation of bean internodes induced ethylene evolution which, in turn, induced the accumulation of high level of IAA. Earlier in ridge gourd early appearance of pistillate flowers was recorded when pruned to six primary branches [17]. Maximum delay in the time for the appearance of 1st staminate and pistillate flower observed in T1, (pruned to main stem only) might be due to the modulation of hormone levels and assimilates translocation.
Table 1: Effect of pruning on changes in endogenous phytohormones levels in the leaves of cucumber cv. ‘Sialkot Selection’. Pruning of lateral branches means removal of lateral branches additional to the number mentioned in the treatments. Measurements for endogenous hormones were made at different physiological stages.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Endogenous GA level (µg/g)</th>
<th>Endogenous IAA level (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Two leaf stage</td>
<td>Flower Initiation stage</td>
</tr>
<tr>
<td>Without pruning</td>
<td>30.9</td>
<td>34.8 d</td>
</tr>
<tr>
<td>Main stem with 1 lateral branch (T2)</td>
<td>30.1</td>
<td>38.4 a</td>
</tr>
<tr>
<td>Main stem with 3 lateral branches (T4)</td>
<td>30.9</td>
<td>35.0 c</td>
</tr>
<tr>
<td>Main stem with 5 lateral branches (T6)</td>
<td>30.0</td>
<td>37.4 b</td>
</tr>
<tr>
<td>LSD value</td>
<td>Ns</td>
<td>0.006890</td>
</tr>
</tbody>
</table>

Means followed by the same letter within same columns do not differ significantly at 5 % level. ** p < 0.01 and * p <0.05.

Fig. 1: Effect of pruning treatments on days to staminate flowers (means of two years with 3 replications per year) and its interaction with endogenous GA level at flower initiation stage. Vertical bars show the standard error means.

Fig. 2: Effect of pruning treatments on days to pistillate flowers (means of two years with 3 replications per year) and its interaction with endogenous IAA level at flower initiation stage. Vertical bars show the standard error means.

Fig. 3: Effect of pruning treatments on staminate flowers (means of two years with 3 replications per year) per plant and its interaction with endogenous GA level at blooming stage. Vertical bars show the standard error means.
Fig. 4: Effect of pruning treatments on pistillate flowers per plant (means of two years with 3 replications per year) and its interaction with endogenous IAA level at blooming stage. Vertical bars show the standard error means.

**Number of Staminate Flowers Plant**\(^{-1}\): Treatments T\(_1\), T\(_2\) and T\(_3\) significantly decreased the total number of staminate flowers plant\(^{-1}\) compared with control (Fig. 3) which could be correlated with lower GA level in T\(_2\) at blooming stage (Table 1). However, maximum number of staminate flowers was observed with the treatments T\(_5\), and T\(_6\) of which results are at par with the control treatment. Greater number of staminate flowers observed in main stem with 5 lateral branches (T\(_5\)) could be due to maximum level of endogenous GA at blooming stage (Table 1). GA is one of the components of florigen. Slight decrease in number of staminate flowers plant\(^{-1}\) in T\(_6\) as compared to T\(_3\) could partially be due to greater allocation of assimilates from vegetative to reproductive parts. Pruning and training (pruned once in every 3-4 months) in mulberry clones showed significant variability in sex expression [18].

**Number of Pistillate Flowers Plant**\(^{-1}\): Treatments T\(_1\) and T\(_5\) significantly increased the total number of pistillate flowers which could be due to higher level of endogenous IAA (Fig. 4) as well as assimilates at blooming stage in treatments of main stem with 2 and 3 lateral branches [16]. The lowest number of pistillate flowers was recorded in the treatment T\(_1\) where more biomass was removed. Contrary to this Folke and Delph [19] reported increased pistillate flower production with physical disturbance in which more biomass was removed. However in most of the previous studies it was reported that mechanical stress (moderate rubbing) in cucumber [20] and pruning in pumpkin [21] increased the number of pistillate flowers.

**Sex Ratio:** The lowest \(\varphi\) to \(\varphi\) flower ratio recorded in pruning treatment of ‘main stem with 3 lateral branches’ (T\(_3\)) could be due to the greater number of pistillate flowers which had a negative correlation \((r=-0.262)\) with sex ratio (Table 2). Akpan and Odejimi [21] also reported the similar results. However, the lower sex ratio observed in pruning treatment of ‘main stem only’ (T\(_1\)) might be attributed to less number of both staminate and pistillate flowers plant\(^{-1}\) (Fig. 5).

**Number of Fruits Plant**\(^{-1}\): T\(_2\) and T\(_4\) treatments had significantly higher number of fruits plant\(^{-1}\) compared with the control (Fig. 6) which could be due to greater pistillate flowers production. A positive significant correlation was observed with number of fruits \((r=0.955)\) and it had a negative correlation with sex ratio \((r=-0.193)\) (Table 2). Highest number of fruits in ridge gourd was also recorded when pruned to six primary branches [17]. Lowest number of fruits plant\(^{-1}\) was recorded in the treatment T\(_1\) which was due to low production of pistillate flowers plant\(^{-1}\).

**Average Fruit Weight:** The treatment T\(_3\) significantly increased the average fruit weight compared with control (Fig. 7) which could be due to higher endogenous GA level at fruiting stage. Maximum single fruit weight in ridge gourd was recorded when pruned to six primary branches [17]. All other treatments had no significant effect on average fruit weight.

**Fruit Yield Plant**\(^{-1}\): Treatments T\(_3\) and T\(_4\) resulted in significant increase in yield plant\(^{-1}\). The maximum fruit yield plant\(^{-1}\) was recorded in T\(_4\) which could be due to greater pistillate flowers production and low GA level at blooming & fruiting stages (Table 1). Previously it was reported that mechanical stress reduced gibberellins content in bean plants [22]. Lowest fruit yield plant\(^{-1}\) recorded in T\(_1\) was due to low production of pistillate flowers and fruits plant\(^{-1}\). A positive significant correlation of fruit yield plant\(^{-1}\) was observed with pistillate flower plant\(^{-1}\) and fruits plant\(^{-1}\) \((r=0.925** \& r=0.972**) respectively. However, a negative correlation
Table 2: Correlation coefficient for the ‘Pruning effect on sex expression and yield in cucumber’

<table>
<thead>
<tr>
<th></th>
<th>Sex ratio</th>
<th>Number of fruits per plant</th>
<th>Fruit yield per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pistillate flower per plant</td>
<td>-0.262</td>
<td>0.955**</td>
<td>0.925**</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>-0.193</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of fruits per plant</td>
<td></td>
<td></td>
<td>0.972**</td>
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</tbody>
</table>

** p < 0.01 and * p < 0.05

Fig. 5: Effect of pruning treatments on sex ratio (means of two years with 3 replications per year). Vertical bars show the standard error means.

Fig. 6: Effect of pruning treatments on fruits per plant (means of two years with 3 replications per year). Vertical bars show the standard error means.

Fig. 7: Effect of pruning treatments on single fruit weight (g) (means of two years with 3 replications per year) and its interaction with endogenous GA level at fruiting stage. Vertical bars show the standard error means.

was recorded with sex ratio (r=-0.083). Highest yield in ridge gourd was also reported when pruned to six primary branches [17]. Higher total yield in Japanese cucumber cv. ‘Hokuho No. 2’ was also recorded with shoot pruning [23]. In a field experiment in ridge gourd, telephone method of trailing with higher dose of fertilizers recorded significantly higher fruit yield compared to other levels of fertilizer with farmers method (without trailing) [24].

Level of Endogenous Hormone at Four Plant Growth Stages: Endogenous GA level increased from two leaves to blooming stage but decreased at fruiting stage in treatment of ‘without pruning’. The level of endogenous GA was affected significantly at flower initiation stage. At flower initiation stage, maximum endogenous GA level was recorded in T₂. At blooming stage, GA level increased as compared to flower initiation stage except T₃.
However, GA level significantly increased in treatment T₄ and decreased in T₁ and T₂ compared to treatment without pruning. During fruiting stage, maximum GA level was recorded in T₂. Endogenous GA level increased from two leaf stage up to blooming stage and decreased at fruiting stage except at T₁ where GA level decreased from blooming stage.

Endogenous IAA level increased from 2 leaf stage till blooming stage where it was at maximum in the control treatments. However in pruning treatments, the level of endogenous IAA was affected significantly at flower initiation, blooming and fruiting stages. At all these growth stages, maximum endogenous IAA level was recorded in T₄. IAA level increased from two leaf stage up to blooming stage and it decreased at fruiting stage.

From this study it was concluded that mechanical stress through pruning treatments of main stem with 3 lateral branches was found best for crop improvement and better yield in cucumber and 61% increase in yield was recorded.

REFERENCES


