Proc. Indian Acad. Sci. (Plant Sci.), Vol. 96, No. 6, December 1986, pp. 509–518.


# Effect of some growth regulators on the leaf architecture of *Tagetes erecta* L.

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MS received 1 October 1985; revised 6 October 1986

**Abstract.** Several leaf deformities viz bilobed leaf segment, asymmetrical leaf base, unsegmented simple leaves etc, accompanied with changes in leaf architecture were observed in *Tagetes erecta* L. plants sprayed with aqueous solutions of different growth substances namely Indole-3-acetic acid, Gibberellic acid, 2,3,5-triiodobenzoic acid and Kinetin. The major changes include the gradual conversion of segmented simple leaf into an unsegmented one owing to 2,3,5-triiodobenzoic acid spray treatment.

**Keywords.** *Tagetes erecta*; leaf architecture; growth substances.

## 1. Introduction

An exploration of the changes in the leaf architecture brought about by various chemical treatments, is an interesting field of study as such studies are neglected. We do not have any information of this type, though several morphological changes in leaf shape and size have been reported due to chemical treatments (Bakale and Kolhe 1978). Krause and Boke (1968) reported 2,3,5-triiodobenzoic acid (TIBA) related thickening of leaf lamina with intensification of leaf colour and some raised intercostal regions giving a wrinkled appearance to soybean leaves of *Glycine max*. In the present paper, *Tagetes erecta* L. of the family Asteraceae was used as investigating material to find out the effects of growth regulators on leaf architecture.

## 2. Materials and methods

Experiments to find out the effects of various concentrations of Gibberellic acid ($GA_3$), TIBA and Kinetin (KN) on leaf architecture of *T. erecta* the seeds of which were procured from France were performed in the Botanical Garden of the University. The plants were raised in unglazed earthenware pots of 12" diameter filled with a mixture of garden soil and farmyard manure (2:1 ratio) and were kept under natural conditions of light and temperature. Each selected concentration of a growth substance was sprayed on 10 plants. Thus the experimental design consisted of 17 sets each with 10 pots. Aqueous solutions having 50, 100, 200 and 500 mg/l of indole-3-acetic acid (IAA), $GA_3$, TIBA and 10, 25, 50 and 100 mg/l of KN were prepared. In all these solutions and distilled water, 2 ml of soapnut extract was added as wetting agent. These spray treatments were started when plants were having 5 internodes. It was repeated for the second time at one week interval. Spray was done on the leaf and stem surfaces untill tiny droplets of the solutions adhered to the surface. Mature leaves which emerged after the foliar spray treatments were plucked, washed and

**Table 1.** Effect of different growth substances on leaf-architecture* in *T. erecta* L.

| Treatment | Concentration (ppm) | Leaf-segment/leaf lamina symmetry | Leaf type | Gland position | Type of venation | Primary vein configuration | Breadth of primary vein ($\mu$) | Secondary vein configuration | Inter-secondary vein | Angle of divergence of 2° on 1° vein | | | Tertiary vein | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Base | Middle | Apex | Angle of origin | Pattern |
| Control | 0 | Symmetrical | Simple (segmented) | Marginal | Pinnate-semicraspedodromous | Straight | 77·69 ± 4·63 | Uniformly curved | Composite | 45·80 ± 2·32 | 56·00 ± 2·43 | 50·00 ± 0·78 | OO | Reticulate |
| IAA | 50 | " | " | " | " | " | 99·90 ± 2·32 | " | " | 40·80 ± 1·04 | 53·33 ± 2·22 | 42·67 ± 1·07 | OO | " |
| | 100 | " | " | " | " | " | 83·25 ± 10·42 | " | " | 53·33 ± 1·03 | 53·33 ± 2·23 | 43·33 ± 1·06 | OO | " |
| | 200 | " | " | " | " | " | 62·14 ± 4·32 | " | " | 37·50 ± 2·32 | 39·00 ± 2·13 | 44·33 ± 0·78 | RO | " |
| | 500 | " | " | " | " | " | 31·64 ± 0·36 | " | " | 43·33 ± 0·92 | 39·66 ± 1·06 | 46·67 ± 0·63 | OO | " |
| $GA_3$ | 50 | " | " | " | " | " | 47·72 ± 0·21 | " | " | 38·00 ± 0·62 | 45·00 ± 1·05 | 48·75 ± 2·35 | OO | " |
| | 100 | " | " | " | " | " | 53·85 ± 0·11 | " | " | 35·00 ± 0·32 | 36·67 ± 0·72 | 46·67 ± 4·62 | OO | " |
| | 200 | " | " | " | " | " | 77·69 ± 1·62 | " | Absent | 32·25 ± 0·10 | 27·50 ± 0·43 | 40·00 ± 3·54 | OO | " |
| | 500 | " | " | " | " | Pinnate-brochidodromous | 93·24 ± 1·69 | Curved | " | 28·05 ± 0·08 | 25·75 ± 0·32 | 36·66 ± 2·43 | OO | " |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TIBA | 50 | " | " | Laminar | Alike-Acrodromous (Suprabasal) | " | 109·89 ± 10·62 | Uniformly curved | Composite | 18·60 ± 0·02 | 32·00 ± 0·06 | 36·66 ± 1·63 | OA | " |
| | 100 | " | " | " | " | " | 103·23 ± 4·62 | " | " | 17·20 ± 0·01 | 30·74 ± 0·78 | 29·67 ± 1·62 | OR | " |
| | 200 | Asymmetrical | Simple (Non-segmented) | " | Alike-Actinodromous (Suprabasal) | " | 129·87 ± 5·67 | Sinuous | Absent | 15·60 ± 0·06 | 28·33 ± 0·52 | 26·26 ± 0·94 | RA | " |
| | 500 | " | " | " | " | " | 146·52 ± 0·92 | " | " | 15·00 ± 1·02 | 19·55 ± 0·45 | 21·57 ± 0·24 | RA | " |
| KN | 10 | Symmetrical | Simple (Segmented) | Marginal | Pinnate-semicraspedodromous | " | 68·29 ± 1·43 | Straight to uniformly curved | Composite | 41·66 ± 1·92 | 33·33 ± 0·32 | 43·33 ± 0·53 | OO | " |
| | 25 | " | " | " | " | " | 79·95 ± 0·23 | Uniformly curved | " | 33·75 ± 0·72 | 46·67 ± 0·06 | 46·33 ± 1·07 | OO | " |
| | 50 | " | " | " | " | " | 84·95 ± 1·23 | " | " | 41·25 ± 0·83 | 48·33 ± 0·72 | 50·00 ± 2·39 | OO | " |
| | 100 | " | " | " | " | " | 92·13 ± 0·78 | " | " | 46·67 ± 1·63 | 44·33 ± 0·63 | 39·33 ± 2·42 | OO | " |

*Data of every character are an average of 10 observations/readings. Angle of origin: O, obtuse; R, right; A, acute.

fixed in FAA. For the cleaning of veins, Johansen's (1940) method was applied. Terminology used in the present paper is of Hickey and Wolfe (1975) and Hickey (1979).

## 3. Observations

These implied only for the leaves which emerged after spray treatment. The most remarkable effects were on the leaves emerged on seventh and eighth nodes (spray done at fifth node stage). The leaves are opposite, simple and petiolate having segmented lamina with symmetrical base except in 200 and 500 ppm of TIBA in which the leaf becomes simple without segments and with slightly asymmetrical base. The leaf segment base is obtuse in control as well as in treated plants. The apex of the leaf segment is acute in control as well as in the treated plants (table 1). In 100 ppm TIBA, the uppermost leaf segment becomes bilobed (figures 1E, F). The leaf segment margin is serrate with regular spacing between the teeth in the control (figure 1A), as well as all concentrations of IAA and KN. However, in $GA_3$ upto 200 ppm, leaf segment margin remains serrate but with irregular spacing between the teeth (figure 1B). The teeth also become narrow and narrowness becomes prominent with increase in concentration of $GA_3$ upto 200 ppm (figure 1B). In 500 ppm $GA_3$, teeth are absent and leaf segment becomes entire (figure 1C). In TIBA treatment, from 200 ppm onwards, leaf segments loose their separate identity, resulting in a gradual conversion of the segmented simple leaf into a non-segmented simple one (figure 1). The margins of non-segmented simple leaf are serrate with irregular spacing (figure 1N). The marginal configuration of these teeth show that they are concave on apical side and convex on basal side in leaf segments of control plants as well in the treated plants with all concentrations of IAA and KN and 100 and 200 ppm of $GA_3$. In 50 and 100 ppm TIBA, the leaf segment marginal teeth became accuminate on apical side and convex on basal side. However, the teeth of the simple non-segmented leaf in 200 and 500 ppm TIBA, appear straight on apical side and convex on basal side. The texture is chartaceous and the position of glands is marginal on the lamina in control and other treatments except of TIBA where glands are laminar (figures 1G–N).

### 3.1 *Major venation pattern*

The pinnate semicraspedodromous type of major venation pattern in control (figure 1A) remains unchanged in all the concentrations of IAA, KN and 50, 100 and 200 ppm of $GA_3$. However, in $GA_3$ 500 ppm it is of pinnate brochidodromous type (figure 1C).

The splitting of mid vein at different heights was observed in leaf segments of 50 and 100 ppm TIBA sprayed plants (figures 1D–F). Therefore, venation pattern was somewhat like suprabasal acrodromous type. In higher concentrations of TIBA, the secondaries of such leaves show gradual fusion and so also the fusion of lobes/leaf-segments (figures 1G–J). Ultimately there was a simple leaf (with some lobes) with entire margin and a single primary vein (figure 1N). As per Hickey (1973) classification, the nearest type of venation pattern in leaves showing transitional stages seems to resemble the suprabasal actinodromous.

The primary vein of the segment in the control leaf is 77·69 $\mu$ thick. In 50 and

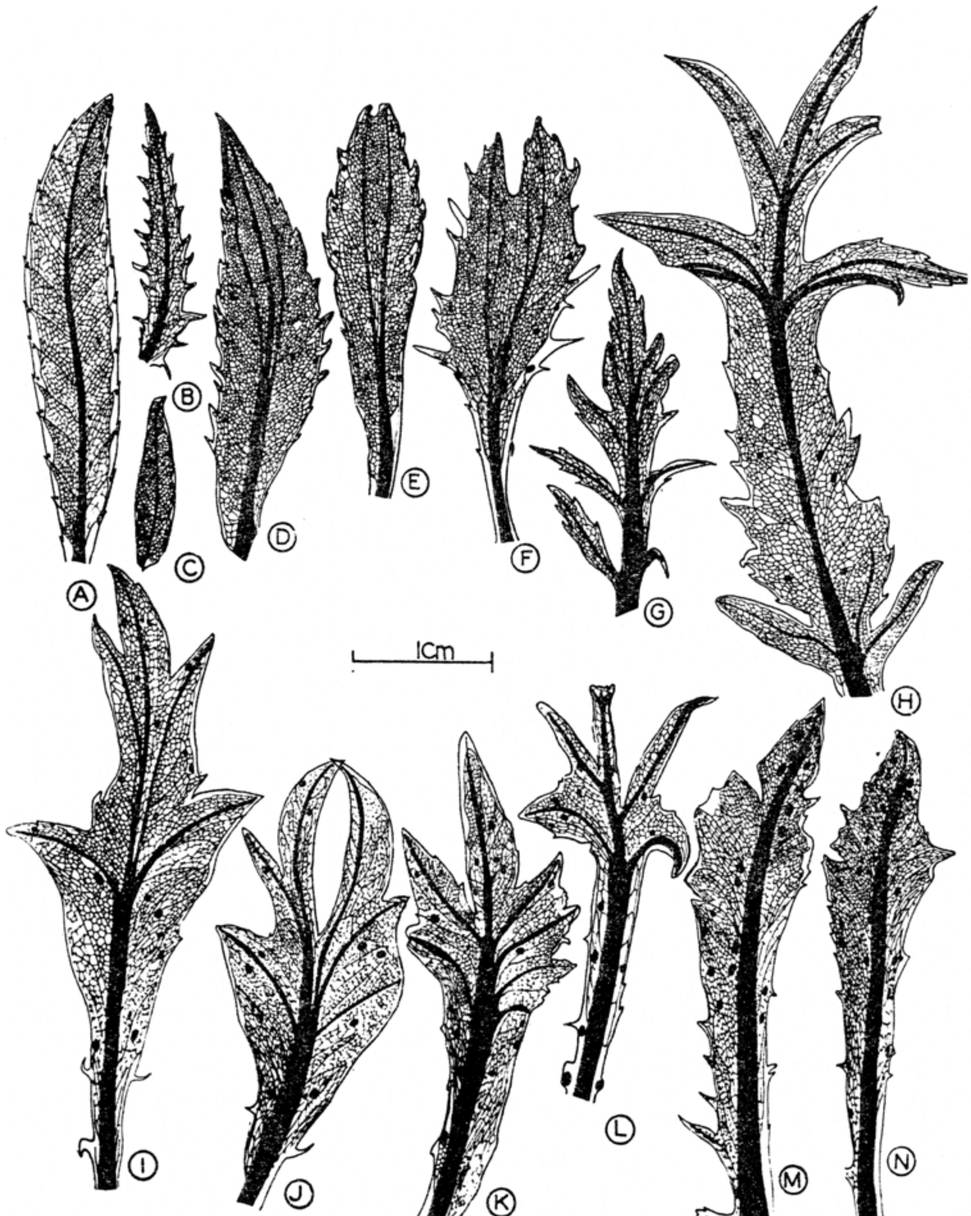


**Figure 1.** Effect of different growth substances on leaf architectural studies. **A–F.** Leaf segment. **A.** Control, showing pinnate semicraspedodromous type of venation, marginal glands and regularly placed serrations at the margin. **B.** $GA_3$ 200 ppm, showing pinnate semicraspedodromous type of venation, marginal glands, narrow teeth with irregular spacing. **C.** $GA_3$ 500 ppm, showing pinnate-brochidodromous type of venation and entire margin. **D.** TIBA 50 ppm, showing the venation pattern alike of suprabasal acrodromous type and irregularly placed serrations at the margin. **E–F.** TIBA 100 ppm, showing the venation pattern alike of suprabasal acrodromous type, irregularly placed serrations at the margin, laminar glands and bilobed apex. **G–N.** Whole leaf of TIBA 200 and 500 ppm, showing the venation pattern alike of suprabasal actinodromous type and gradual loss of segmentations. Glands are laminar.

**Table 2.** Effect of different growth substances on minor venation pattern* of *T. erecta* L. leaf/leaf segment.

| Treatment | Concentration (ppm) | Maximum higher order venation | Higher order venation resolution | Areole shape | Areole frequency ($mm^{-2}$) Base | Areole frequency ($mm^{-2}$) Middle | Areole frequency ($mm^{-2}$) Apex | Size of Areoles ($\mu$) Base | Size of Areoles ($\mu$) Middle | Size of Areoles ($\mu$) Apex | Number of veinlets per areole |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | 4°, 5° | Reticulum | Quadrangular to polygonal | 21·66 ± 1·24 | 16·01 ± 1·04 | 25·42 ± 2·31 | 178·69 × 59·94 ± 10·32 ± 2·34 | 178·99 × 99·90 ± 10·33 ± 2·32 | 120·71 × 76·59 ± 10·23 ± 9·78 | 0–2 |
| IAA | 50 | 4°, 5° | " | " | 26·37 ± 1·22 | 26·36 ± 1·07 | 28·72 ± 2·46 | 119·88 × 66·83 ± 10·41 ± 2·46 | 112·02 × 69·93 ± 9·74 ± 2·32 | 105·73 × 61·17 ± 2·34 ± 2·12 | 0–2 |
| | 100 | 4°, 5° | " | " | 25·37 ± 0·63 | 33·89 ± 2·32 | 31·37 ± 3·12 | 120·98 × 69·93 ± 9·77 ± 1·92 | 88·78 × 68·80 ± 4·37 ± 1·62 | 86·58 × 59·94 ± 1·75 ± 1·62 | 0–1 Rarely 2 |
| | 200 | 4°, 5° | " | " | 33·89 ± 1·32 | 39·55 ± 1·94 | 32·64 ± 2·53 | 76·59 × 61·94 ± 0·74 ± 0·32 | 74·36 × 59·94 ± 3·56 ± 2·91 | 85·45 × 63·27 ± 2·34 ± 1·04 | 0–1 |
| | 500 | 4°, 5° | " | " | 42·67 ± 0·64 | 43·31 ± 0·76 | 35·78 ± 1·42 | 65·47 × 45·49 ± 0·32 ± 0·14 | 66·60 × 45·49 ± 0·62 ± 1·32 | 68·27 × 59·94 ± 2·78 ± 1·06 | 0–1 |
| $GA_3$ | 50 | 4°, 5° | " | " | 31·64 ± 0·69 | 33·89 ± 2·62 | 41·43 ± 1·36 | 95·24 × 44·86 ± 0·93 ± 0·12 | 78·82 × 76·59 ± 0·34 ± 0·62 | 88·48 × 44·39 ± 3·44 ± 1·92 | 0–2 |
| | 100 | 4°, 5° | " | " | 26·87 ± 0·52 | 30·13 ± 3·42 | 36·40 ± 2·62 | 111·56 × 41·63 ± 2·34 ± 1·02 | 82·12 × 66·00 ± 1·46 ± 1·23 | 104·33 × 44·39 ± 10·96 ± 1·97 | 0–2 |
| | 200 | 3°, 4° rarely 5° | " | " | 15·06 ± 1·23 | 14·12 ± 0·41 | 20·07 ± 1·06 | 183·15 × 89·91 ± 10·16 ± 0·78 | 143·19 × 73·26 ± 10·98 ± 10·78 | 143·19 × 51·05 ± 4·62 ± 1·32 | 0–3 |
| | 500 | 3°, 4° | " | Triangular to pentangular | 8·47 ± 0·98 | 9·42 ± 0·62 | 9·26 ± 0·04 | 249·75 × 76·59 ± 10·52 ± 0·94 | 233·93 × 107·39 ± 6·94 ± 5·32 | — | Mostly 0 rarely 1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| TIBA | 50 | 4°, 5° | " | Quadrangular to polygonal | 23·07 ± 0·97 | 31·37 ± 0·21 | 39·54 ± 2·34 | 213·12 × 48·82 ± 10·78 ± 0·23 | 94·14 × 45·79 ± 4·37 ± 2·34 | 72·47 × 42·16 ± 0·32 ± 0·41 | 0–2 |
| | 100 | 4°, 5° | " | " | 26·12 ± 1·63 | 35·14 ± 0·22 | 49·31 ± 1·94 | 181·72 × 50·85 ± 7·42 ± 1·43 | 83·78 × 19·38 ± 1·95 ± 1·20 | 74·93 × 55·77 ± 0·41 ± 0·76 | 0–1 |
| | 200 | 4°, rarely 5° | " | " | 28·25 ± 1·78 | 45·67 ± 4·32 | 49·31 ± 1·93 | 114·05 × 59·12 ± 0·92 ± 0·12 | 82·42 × 40·86 ± 0·32 ± 0·41 | 82·42 × 38·29 ± 1·32 ± 1·42 | Rarely 1 in areoles of leaf-margin |
| | 500 | 4° | " | Triangular at base quadrangular to polygonal at middle and apex | 32·40 ± 1·92 | 44·69 ± 2·41 | 45·19 ± 0·32 | 92·01 × 59·94 ± 0·03 ± 0·92 | 67·43 × 62·40 ± 0·52 ± 0·76 | 84·15 × 35·60 ± 1·41 ± 1·32 | 0 |
| KN | 10 | 4°, rarely 5° | " | Quadrangular to polygonal | 37·66 ± 1·94 | 38·29 ± 0·32 | 35·78 ± 1·32 | 120·98 × 59·94 ± 10·23 ± 2·12 | 103·23 × 51·94 ± 2·46 ± 1·32 | 129·87 × 39·96 ± 0·46 ± 0·92 | 0–2 |
| | 25 | 4°, rarely 5° | " | " | 30·13 ± 2·32 | 30·13 ± 0·16 | 37·19 ± 2·63 | 141·53 × 43·29 ± 10·42 ± 4·32 | 139·86 × 55·48 ± 1·42 ± 2·32 | 91·01 × 93·24 ± 0·92 ± 0·76 | 0–2 |
| | 50 | 4°, 5° | " | " | 39·54 ± 2·96 | 35·78 ± 0·14 | 48·32 ± 3·42 | 81·50 × 79·92 ± 9·32 ± 2·92 | 117·38 × 42·46 ± 1·46 ± 1·24 | 63·27 × 36·63 ± 1·32 ± 1·42 | 0–1 Rarely 2 |
| | 100 | 4°, 5° | " | " | 43·31 ± 3·42 | 56·49 ± 1·94 | 60·26 ± 4·63 | 63·27 × 42·16 ± 3·57 ± 0·92 | 59·11 × 38·29 ± 1·24 ± 1·32 | 51·05 × 41·06 ± 2·32 ± 0·09 | 0–1 |

*Data of every character are an average of 10 observations/readings.

100 ppm it is slightly thicker i.e. 99·90 and 83·25 $\mu$) whereas in 200 and 500 ppm IAA it is thinner (i.e. 62·14 and 31·64 $\mu$). The primary vein is 47·72 $\mu$ thick in 50 ppm $GA_3$ and 53·85 $\mu$ thick in 100 ppm $GA_3$, but in higher concentrations of $GA_3$ it is either equal or more than the thickness of the primary vein in control. In KN, primary vein thickness is slightly more than that in control while in TIBA, primary veins are massive and prominent and the thickness being almost double the control (table 1).

The course of secondary vein is observed to be uniformly curved in IAA, $GA_3$, KN and lower TIBA concentrations. However, in higher concentrations of TIBA secondaries are sinuately curved. Intersecondary veins are observed in control as well as in all treatments except in 200 and 500 ppm concentrations of $GA_3$ and TIBA. The intersecondaries are composite being of coalesced tertiary vein segments (table 1).

The angle of origin of the secondary vein (table 2) is less than right angle i.e. acute in the control. However, the acuteness pronounced in TIBA and $GA_3$ treatments whereas it remains almost insignificantly affected in IAA and KN treatments. The tertiary veins show random-reticulate pattern anastomosing with the other tertiaries or secondaries. The predominant angle of origin of the tertiary vein is more than 90° being obtuse-obtuse type on both the admedial and exmedial sides. It remains the same in all treatments except in TIBA where on admedial and exmedial sides it is obtuse-acute in 50 ppm, obtuse-right in 100 ppm and right-acute in 200 and 500 ppm. However, the pattern of tertiary veins is random reticulate type i.e. the tertiary veins anastomose with other tertiary veins or secondaries.

### 3.2 *Minor venation pattern*

It is constituted by the veins of the quaternary and quinternary veins. Generally, quinternary veins are the highest order veins observed in the leaf segments of *Tagetes erecta*. However, quaternary veins appear to be of the highest order, in leaves of the plants sprayed with 500 ppm of $GA_3$ and TIBA.

A great variation in shape and size of the areoles in the treated and control plants is also evident (table 2). They are quadrangular, pentangular and polygonal in leaf segments of the control and all treatments except 500 ppm $GA_3$ and TIBA. In 500 ppm $GA_3$ sprayed plants, triangular to pentangular areoles are dominant while in 500 ppm TIBA sprayed plants areoles are mostly triangular at base of leaf, but quadrangular to pentangular at middle and apex of leaf.

The sizes of areoles decrease as compared to those in control in apical, middle and basal parts of leaf segment treated with IAA and KN and this decrease becomes more pronounced with increase in the concentration. The areoles of middle and apical region in the leaf or leaf segment of TIBA and $GA_3$ treated plants are also smaller than control. However, at basal side they are smaller than control only in lower concentrations of $GA_3$ and higher concentrations of TIBA. As compared to the control the frequency of areoles is more in all treatments except in higher concentrations of $GA_3$. The minimum areole frequency 8·47 and 9·42 in basal and middle region of leaf segment respectively is in 500 ppm $GA_3$ treatment. The maximum areole frequency is in 100 ppm kinetin where it is 43·31, 56·49 and 60·26 in basal, middle and apical portion of leaf segment respectively (table 2).

The number of vein-lets in an areole is variable even in the same leaf segment. In control, usually one or two veinlets enter the areoles but sometimes they are devoid of any veinlet. The condition is almost the same in lower concentrations of all the treatments. However, in higher concentrations of IAA and kinetin, usually one veinlet per areole is observed and in some areoles even this is absent. In 200 ppm TIBA mostly the areoles are devoid of any veinlet, but in some areoles on the marginal side sometimes single veinlet is present. In 500 ppm TIBA almost all the areoles are devoid of any veinlet. In 500 ppm $GA_3$ also, veinlets are mostly absent in the areoles (table 2).

Veinlets in control are linear or curved with once or twice branching. In IAA, TIBA and KN they are simple, linear or curved and unbranched. However, in lower concentrations of $GA_3$ they are sometimes once or rarely twice branched.

The marginal ultimate venation forms loops in treated and control plants indicating that it is unaltered by any chemical treatment employed.

## 4. Discussion

The normal plants of *Tagetes erecta* bear simple, petiolate, symmetrical, segmented opposite leaves. While those treated by 200 and 500 ppm of TIBA develop simple ones with a slight asymmetrical base, and display several transitional stages with loss of segmentation. The leaf-segment margins are serrate with regular spacing between the teeth, in the control, IAA and KN sprayed plants. But in $GA_3$ (upto 200 ppm), leaf-segments margins remain serrate but with irregularly spaced and narrower teeth. In 500 ppm $GA_3$ teeth are absent and leaf-segment becomes entire. In TIBA 200 and 500 ppm treatments, leaf-segments lose their separate identity, through progressive reduction resulting in a simple acute leaf.

Hickey and Wolfe (1975) identified the order Asterales, to which *T. erecta* belongs, with pinnatified leaves having pinnate brochidodromous type of venation. However, in the leaf-segments of the leaves of control and IAA (all concentrations), $GA_3$ (upto 200 ppm) and KN (all concentrations) treated plants, pinnate semicraspedodromous type of venation pattern is recognized. It is characterized by a single primary vein (mid vein) from which secondary veins arise alternately on both sides and divide just within the margin with one of the branches terminating, whereas the other joining the superadjacent secondary. However, the leafsegments of 500 ppm $GA_3$ treated plants show pinnate brochidodromous type of major venation pattern in which secondary veins do not terminate at the margin but join together in a series of prominent arches. In TIBA 50 and 100 ppm treatments, the leafsegments show splitting of the mid-vein at different heights from the base. The major venation, thus resembles somewhat to the acrodromous suprabasal type. In leaves of 200 and 500 ppm of TIBA sprayed plants, leafsegments lose their demarcation. Although, in 200 ppm, there is some segmentation with irregular spacing, in the 500 ppm, segmentation is almost nil with only a few serrations here and there on the leaf margins. After the spray, the first 3 leaves show transitional stages of collapsing. The simple entire leaves are fourth and onwards after the spray treatment.

With this loss of segmentation, the problem is how to compare such leaves with those of the control? Should the whole leaf or only one of its segments be taken for comparison? For convenience in comparison and description the whole segmented control leaf has been taken as the unit of comparison with the unsegmented leaf, in the following paragraph.

The venation of leaves of 200 and 500 ppm TIBA treatments shows a gradual collapse of the secondaries (which may be primaries of the leafsegments) from base upwards and so also the collapse of the segments/lobes which ultimately leads to a single simple leaf with unsegmented lamina and with a single massive primary vein. Although the transitional stages do not show resemblance with any of the venation patterns given by Hickey (1973), the nearest type seems suprabasal actinodromous. Furthermore, the leaf segment/leaf of TIBA sprayed plants show laminar distribution of glands in contrast to marginal distribution in leafsegments of control and rest of the treatments.

A wide variation in the shape and frequency of the areoles due to treatment with different growth substances supports the view that the size of the areole cannot serve as a useful taxonomic character. Such a view was presented earlier by Banerji and Das (1972) and Sehgal and Paliwal (1974). The growth substances also cause variations in the angle of divergence of the secondary veins.

## Acknowledgements

One of the authors (Vidhu) is grateful to University Grants Commission, New Delhi for a fellowship.

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