

Effect of Triiodobenzoic Acid on Broomrape (*Orobanche ramosa*) Infection and Development in Tomato Plants

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Branched broomrape (*Orobanche ramosa*) is a holoparasitic flowering plant that attaches to the root of its host, green plant, by means of a specialized structure known as haustorium. Following successful contact and penetration on susceptible plant root, complex tissue of *Orobanche* cells is formed which is known as the tubercle. Newly formed tubercles contain high activity of indole-3-acetic acid (IAA). Triiodobenzoic acid (TIBA), as an inhibitor of IAA polar transport, was utilized to investigate the supply and requirement of auxin to the developing *O. ramosa* on tomato plant. There was no significant reduction in the incidence of *O. ramosa* per pot of different TIBA treatments. However, infection severity in terms of the number of *O. ramosa* shoots that emerged per plant and number of attachments per plant root system were significantly reduced by 60% and 45% on TIBA treated plants, respectively. Histological studies revealed conspicuous delay in the initiation of xylem vessel differentiation inside tubercles of the TIBA treated tomato plants. Also, differentiated vessels showed thinner secondary wall deposition, and improper alignment within bundles inside those tubercles. They were wider and shorter in diameter in comparison to those of untreated plants. These findings were attributed to the short supply of IAA required for normal development, and to the xylem vessel differentiation of *O. ramosa* tubercles on infected tomato. Hence, this parasitic flowering plant seems to depend upon its host in its requirements for IAA, in a source to sink relationship.

Keywords : Indole-3-acetic acid, *Orobanche ramosa*, polar transport inhibition, xylem differentiation

Orobanche species are parasitic flowering plants that contain no chlorophyll and lack true roots (Foy, 1981; Jain and Foy, 1989; Parker and Riches, 1993). Therefore, it poses a serious threat to many agricultural crops, particularly to

several dicotyledonous plant families, as this parasite grows and develops totally on the expense of its host plant through its water and photosynthesis products (Foy et al., 1989). Morphological and anatomical developments of *Orobanche* are well investigated (Parker and Riches, 1993). However, little is known about the involvement of hormones and growth regulators during those developmental processes. Therefore, further understanding of these aspects may contribute to a better understanding of host-parasite interaction and possibly to the development of effective control measures.

Plant hormones regulate the growth and development in green plants (Davies, 1995), which are potential hosts of parasitic seed plants. Hormones may also be similarly involved in *Orobanche* development (Magnus et al., 1982). Indole-3-acetic acid (IAA) is widely present in plant tissues (Davies, 1995; Muday and Haworth, 1994) and is essentially involved in the differentiation of xylem vessels (Davies, 1995; Muday and Haworth, 1994). This hormone was also documented to be present in the tissue of *O. ramosa* tubercles in tomato (Sekkat et al., 1994). A newly formed tubercle is made of complex tissue, which is a mixture of the host and parasite tissues (Parker and Riches, 1993). As tubercle grows older, it differentiates xylem vessels inside its core in order to establish vascular connection with the host plant (Parker and Riches, 1993). Hence, it is hypothesized in this present investigation that host plants may act as source of IAA necessary for the development of tubercles and for the differentiation of functional xylem vessels inside *Orobanche* tubercles.

Materials and Methods

Plant materials. Tomato seeds (*Lycopersicon esculentum* var. Rio Grand) were obtained from the Center for Agricultural Researches at the Jordan University of Science and Technology. *Orobanche ramosa* seeds were collected from infected tomato fields in the Jordan valley (Al-Ghour region in northern Jordan). Two-week-old tomato seedlings were transplanted into plastic pots (20 cm) containing soil mix made of silt-loam soil sup-

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plemented with sand at the ratio of 2:1 (v/v). *O. ramosa* seeds (1000 seeds/plant) were sprinkled on the root system inside the planting holes.

Inhibition of IAA polar transport by Triiodobenzoic acid (TIBA). A solution of TIBA in lanolin at a concentration of 10 mg/g (w/w) was used in pasting a 10-mm long ring on the base of tomato seedlings close to the soil surface 2 weeks and 4 weeks from transplanting date. Plants which were either inoculated or non-inoculated with *O. ramosa* seeds were treated with TIBA. Untreated plants and plants pasted with lanolin alone without TIBA served as control. All treatments were replicated six times and the experiments were repeated twice, once under greenhouse condition ($24 \pm 2^\circ\text{C}$) day and night, and the second time inside a growth cabinet under laboratory condition with continuous artificial light intensity of 421 cd sr m^{-2} and 25°C . Incidence of infection was determined in terms of *O. ramosa* shoot emergence per pot, and infection severity was measured as the number of *O. ramosa* shoots per plant, and number of tubercles per plant root system.

Experiments were harvested about 7 weeks from transplanting date, when 50-60% of untreated plants (TIBA not applied) grown in *O. ramosa*-infested pots contained one or more emerged *O. ramosa* shoots.

Comparative histological development of *Orobanche* tubercles in TIBA-treated and untreated tomato plants. Tubercles of *O. ramosa* in small nodule and spider-shaped stages were collected and immediately placed in fixative-preservative solution made of 70% ethanol, 40% formaldehyde, and glacial acetic acid (FAA) at the ratio of 8.5:1:0.5, respectively. Preserved tubercles were dehydrated, infiltrated, embedded in paraffin wax molds, and sectioned into 10 μm serial sections which were mounted and stained by safranin-methylene green double stain. Permanent slides were secured in D.P.X mounting material and examined under dissecting and light microscopes. Histological differences were assessed in terms of xylem procambial cell development and

their alignment within tubercles, and the organization of xylem vessels. Morphology and integrity of those vessels were investigated as well.

Quantitative analysis of IAA levels in *Orobanche* tubercles of TIBA-treated and untreated tomato plants. *Orobanche* tubercles of TIBA-treated and untreated plants were analyzed for their IAA levels. About 200 mg of tubercle tissue at the same stage of development from both treatments were analyzed for their IAA contents utilizing competitive Enzyme Linked Immuno-Sorbent Assay (ELISA) (Weiler et al., 1981). Tubercles were extracted according to the procedure described by Liu et al. (1998). The dried fraction of each treatment was re-dissolved in 200 μl absolute methanol, and then methylated with trimethylsilyldiazomethane (TMSCHN₂) for 24 hours with continuous shaking (Hashimoto et al., 1981). The methylated fraction of each stage was dried by flash evaporation at 35°C , and then re-dissolved in 1ml Trizma Base Sodium azide (TBS) buffer (pH 7.5). IAA in tissues of each treatment was quantified by competitive ELISA (Phytdetek Kit, Agdia, Indiana, USA) according to the manufacturers instructions, and methylated-IAA was used as standard.

Results and Discussion

Measurements of the shoot and root fresh weight, length, and dry weight showed that the application of TIBA (10 mg/g lanolin) on non-inoculated plants significantly reduced the dry weight of the shoot system, whereas, no significant reduction in the other parameters was noted (Table 1). The application of TIBA (10 mg/g lanolin) on inoculated plants led to a noticeable improvement in the vegetative growth of those plants in contrast with that of the untreated inoculated plants (Table 1). Reed et al. (1998) reported an adverse effect of TIBA on the seminal root development in *Arabidopsis* using 100 μM treatment. Incidence of *O. ramosa*

Table 1. Effect of TIBA (10 mg/g lanolin) on shoot and root length, fresh weight, and dry weight of tomato plants inoculated with *O. ramosa* and non-inoculated control plants

| Treatment | Tomato plant grown in potted soil | | | | | | | | | | | |
|--------------------------|-----------------------------------|-------|--------|-------|--------|-------|-------------|-------|--------|-------|-------|-------|
| | Non-inoculated | | | | | | Inoculated | | | | | |
| | Length (cm) | | FW (g) | | DW (g) | | Length (cm) | | FW (g) | | DW(g) | |
| | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot |
| Control 1 ^a | 38.1 | 24.1 | 4.6 | 5.9 | 0.52 | 1.23 | 20.8 | 14.4 | 2.25 | 3.3 | 0.21 | 0.52 |
| Control 2 ^b | 38.1 | 23.9 | 4.5 | 5.8 | 0.96 | 1.21 | 16.9 | 13.0 | 1.8 | 2.3 | 0.17 | 0.33 |
| Treatment 1 ^c | 35.4 | 24.3 | 3.1 | 6.1 | 0.50 | 0.98 | 23.2 | 14.4 | 2.7 | 3.3 | 0.25 | 0.65 |
| Treatment 2 ^d | 46.2 | 24.1 | 5.7 | 4.9 | 0.78 | 0.98 | 32.3 | 17.1 | 3.4 | 4.2 | 0.32 | 0.66 |
| LSD ($p = 0.05$) | 10.85 | 3.99 | 2.45 | 1.87 | 0.27 | 0.33 | 10.85 | 3.99 | 2.45 | 1.87 | 0.27 | 0.33 |

FW: fresh weight

DW: dry weight

^aNon-inoculated with *O. ramosa* seeds and received no treatment of lanolin or TIBA.

^bNon-inoculated with *O. ramosa* seeds but treated with lanolin only.

^cInoculated with *O. ramosa* seeds and treated with lanolin-TIBA mixture (10 mg/g) at 2 weeks after transplanting.

^dInoculated with *O. ramosa* seeds and treated with lanolin-TIBA mixture (10 mg/g) at 4 weeks after transplanting.

All values are averages of six replications.

Table 2. Effect of TIBA (10 mg/g) on the number of *Orobanchae* shoots and tubercles per tomato plant

| Treatment | Number of <i>O. ramosa</i> shoots and tubercles on the roots of tomato plants | | | |
|--------------------------|---|-----------|------------|-----------|
| | Non-inoculated | | Inoculated | |
| | Shoots | Tubercles | Shoots | Tubercles |
| Control 1 ^a | 0.00 | 0.00 | 1.16 | 4.27 |
| Control 2 ^b | 0.00 | 0.00 | 1.08 | 4.01 |
| Treatment 1 ^c | 0.00 | 0.00 | 0.29 | 2.42 |
| Treatment 2 ^d | 0.00 | 0.00 | 0.17 | 2.73 |
| LSD ($p = 0.05$) | 0.62 | 0.92 | 0.62 | 0.92 |

^aNon-inoculated with *O. ramosa* seeds and received no treatment of lanolin or TIBA.

^bNon-inoculated with *O. ramosa* seeds but treated with lanolin only.

^cInoculated with *O. ramosa* seeds and treated with lanolin-TIBA mixture (10 mg/g) at 2 weeks after transplanting.

^dInoculated with *O. ramosa* seeds and treated with lanolin-TIBA mixture (10 mg/g) at 4 weeks after transplanting.

All values are averages of six replications.

infection in terms of the emergence of one or more shoots per pot was not prevented by the application of 10 mg/g TIBA in lanolin paste (Table 2).

However, disease severity measured in terms of the number of emerged, as well as non-emerged *O. ramosa* shoots plus the number of tubercles per plant was significantly lower in the root systems of TIBA-treated plants (Table 2). These results suggest that IAA from the host plant, tomato, is needed at the initial infection until the onset of successful attachment of *O. ramosa*, and the subsequent invasion of root cortex leading to tubercle formation. This was evident by the significant reduction in the number of tubercles that developed on the TIBA-treated plants. This significant reduction in the number of *O. ramosa* shoots and tubercles may reduce the inoculum of this parasite in the soil. Moreover, the importance of IAA in the host-parasite interaction is further substantiated by the results of the histological analysis (Fig. 1). It showed the



Fig. 1. Procambial xylem cells represented by elongated parenchyma cells (EP) inside *Orobanchae* tubercle at stage of development. Triiodobenzoic acid (TIBA) was not applied in A, but was applied in B and C at 2 weeks and 4 weeks after transplanting, respectively. 200X

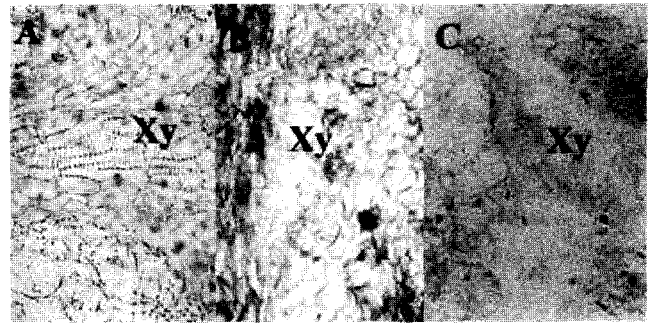


Fig. 2. The differentiation of xylem elements (Xy) in *Orobanchae* tubercle. Triiodobenzoic acid (TIBA) was not applied in A, but was applied in B and C at 2 weeks and 4 weeks after transplanting, respectively. 200X

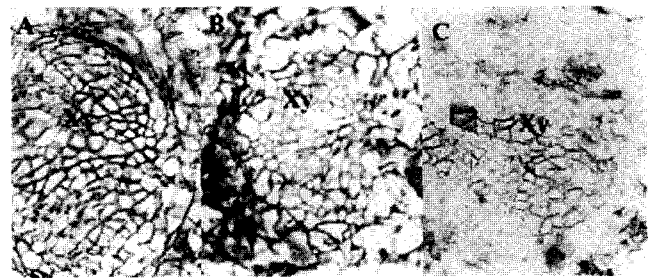


Fig. 3. Cross-section in *Orobanchae* tubercle showing secondary wall deposition. Triiodobenzoic acid (TIBA) was not applied in A, but was applied in B and C at 2 weeks and 4 weeks after transplanting, respectively. 200X (Xy: xylem elements).

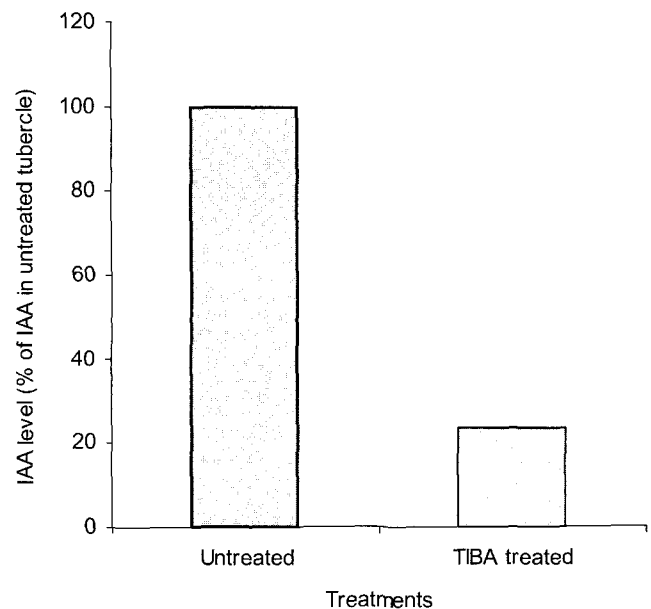


Fig. 4. Level of IAA in *Orobanchae* tubercles in the tomato plants untreated and treated with TIBA (10 mg/g lanolin) as detected by competitive ELISA. The level of IAA in the untreated control was expressed as 100%.

failure of vascular tissue differentiation in the early stages of *O. ramosa* development on the roots of TIBA-treated plants compared to those of untreated control. Moreover, abnormal wider and shorter xylem vessels are shown in *O. ramosa* tubercles as a result of TIBA application (Fig. 2). These abnormal vessels are not aligned, and are discontinuously arranged within, which are supposed to appear as bundles. They show thinner secondary wall deposition than the control (Fig. 3). Such findings are consistent with previous studies, which found that xylem differentiation requires high concentration of IAA (Aloni, 1995). Also, inhibitors of the polar transport of IAA lead to abnormally differentiated xylem vessels (Mattsson et al., 1999). Therefore, it could be postulated that the lack of xylem vessel continuity at the early stages of *O. ramosa* on TIBA-treated tomato evidently was due to shortage in IAA concentrations (Fig. 4). Hence, this parasitic flowering plant seems to depend upon its host in its requirements for IAA, in a source to sink relationship.

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