Scientia Horticulturae 162 (2013) 76–81

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of indole-3-acetic acid and auxin transport inhibitor on auxin distribution and development of peanut at pegging stage

Qiong Peng, Huiqun Wang, Jianhua Tong, Mohammed Humayun Kabir, Zhigang Huang, Langtao Xiao*

Hunan Provincial Key Laboratory of Phytohormones and Growth Development, Hunan Provincial Key Laboratory for Crop Germplasm Innovation and Utilization, College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha 410128, Hunan, People's Republic of China

ARTICLE INFO

Article history:
Received 26 March 2013
Received in revised form 21 July 2013
Accepted 23 July 2013

Keywords:
Indole-3-acetic acid
Auxin transport inhibitor
Pegging stage
Peanut

ABSTRACT

This study was conducted to determine the effects of indole-3-acetic acid (IAA) and auxin polar transport inhibitor 2,3,5-triiodobenzoic acid (TIBA) on the growth, photosynthetic characteristics of leaves and IAA contents in different parts of peanut cv. Zhonghua 4 at pegging stage. It was found that spraying IAA resulted in an increase in IAA contents of different parts, the photosynthetic rate, and the growth of stem, peg and flower at pegging stage. Treatment with TIBA increased the first branch length and branch number per plant by 6.83% and 15.34%, while the stem height, peg length, flower number and peg number per plant were significantly inhibited by 25.08%, 29.52%, 33.18% and 30.14%. The average weights of single pod and the yield per pot with auxin and TIBA treatments were distinctly higher than the control. Pegging rate and net photosynthesis rate were enhanced by 4.56% and 12.67%, respectively, but intercellular CO_2 concentration (C_i) was decreased than the control by 8.06% under 10 μM TIBA treatment. The IAA contents of stems and leaves treated with 10 μM TIBA were also significantly increased. These results suggest that auxin, along with its transport and distribution, control the growth of peanut at pegging stage, which may contribute to higher yield through enhanced pod development.

1. Introduction

Peanut (*Arachis hypogaea* L.) is one of the principal economic crops as a resource for edible oil and protein, which has one of the most fascinating growth habits in the plant kingdom, i.e. the peanut plant produces flowers aerially, but it is capable of "sowing" its own seeds underground. After pollination, fertilized ovaries of above-ground flowers develop into gynophores, commonly known as pegs (Haro et al., 2011). The peg displays some of the properties of both root and shoot as well as being a reproductive organ (Jacobs, 1947). For peg growth, many physiological changes in plant like phyto-hormonal accumulation and distribution take place throughout its development (Moctezuma, 2003).

Indole-3-acetic acid (IAA) is the main phytohormone of the auxin group in plants which has profound effects on plant growth and development as well as other physiological processes (Zhao, 2010). Previous studies have shown that IAA plays a major role in the growth and development of the peanut pegs (Shushu and Cutter, 1990b; Moctezuma, 1999). The research showed that IAA was localized within the tissues of vertically oriented and gravi-stimulated pegs (Moctezuma and Feldman, 1999). A crucial aspect of auxin action is its graded distribution which depends on local auxin biosynthesis (Stepanova et al., 2008; Ikeda et al., 2009) and directional, intercellular auxin transport (Petrásek et al., 2006; Grunewald and Friml, 2010). The polar auxin transport has an essential role in most auxin-regulated processes and is mediated by homologues of the ATP Binding Cassette subgroup B (ABCB) multiple drug resistance transporters (Geisler et al., 2005), by auxin influx proteins of the AUX1/LAX family (Swarup et al., 2008) and by PIN-formed (PIN) auxin efflux proteins (Petrášek and Friml, 2009).

Auxin efflux inhibitor such as 2,3,5-triiodobenzoic acid (TIBA) is thought to specifically block the basipetal movement of IAA from its site of synthesis in the shoot apex to sites of action further down the stem (Lomax et al., 1995). This compound has significantly contributed to the present knowledge about auxin efflux transporters and their involvement in the control of physiological and developmental processes in plants (Morris et al., 2004; Luo et al., 2012). Being an auxin transport inhibitor, TIBA effectively blocks auxin transport by binding to the regulatory site of an auxin efflux carrier complex. Therefore PIN cycling and the movement from endosomal compartments to the plasma membrane was inhibited by the auxin transport inhibitor TIBA (Friml, 2010). Previous study

* Corresponding author. Tel.: +86 731 84635261; fax: +86 731 84635261.
E-mail addresses: langtaotxiao@phytohormones.com, langtaoxiao@163.com (L. Xiao).

0304-4238/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scienta.2013.07.027

indicated that low concentration of TIBA decreased the response of somatic embryogenesis and higher levels being inhibitory for somatic embryogenesis in peanut (Venkatesh et al., 2009), but neither is known about the IAA production in peanut plant at pegging stage, nor the relationship between auxin transport and the physiological changes taking place in this critical stage. Therefore, it is important to reveal the correlation between IAA polar transport and the development of peanut at pegging stage from the view of whole-plant physiology.

In this study, our experimental aims were to examine the effects of foliar spraying auxin and auxin polar transport inhibitor TIBA on the growth of nutritive and reproductive organs, the photosynthetic capacity and IAA distribution in peanut plant at pegging stage.

2. Materials and methods

2.1. Plant material and experimental design

Pot grown peanut (*A. hypogaea* L.) cv. Zhonghua 4 was used as the experimental material. The experiments and analysis were respectively conducted at the experimental farm of Hunan Agricultural University and Hunan Provincial Key Laboratory of Phytohormones and Growth Development. Plants were grown in cylindrical plastic pots (30 cm diameter × 26 cm height) with a combination of coarse sand, laterite and paddy field soil (4:4:1, v/v/v). Three seeds were sown in each pot. All pots were properly cared according to conventional field management measures.

Indole-3-acetic acid and 2,3,5-triiodobenzoic acid were prepared at 0.1 mol L^{-1} in dimethyl sulfoxide (DMSO). A solution with equivalent DMSO was used as the control. 1×10^{-5} mol L^{-1} IAA and TIBA treatments were performed three times on 3, 6 and 9 days after flowering, respectively. Plants were foliar sprayed until considerable run-off on the leaf surface occurred at early morning. During the treatments, the base of each plant was covered with a layer of thin and transparent plastic film which was removed next morning. Four pots of peanut plants were employed as one treatment. The entire experiment was performed according to a complete randomized design (CRD). Each treatment was replicated three times.

2.2. Sampling and measurements

Peanut development was divided into nine stages of reproductive growth as described by Boote (1982). The pegs of the first node, roots, stems, leaves and flowers from peanut plants were collected at pegging stage (i.e. the 20th day after the treatments). Collected samples were immediately wrapped in aluminum foil and frozen in liquid nitrogen, then placed into a sealed plastic bag and stored at −80 °C until the extraction, purification and determination of IAA.

The data for the number of branches per plant, number of flowers per plant, number of pegs per plant were collected as well as the lengths of stems, the first lateral branches and pegs from the first node. At mature stage (i.e. the 50th day after treatments), the yield per pot and the average dry weight of single pod from each treatment were recorded. Pegging rate (%) was computed by using the following equation.

$$\text{Pegging rate}(\%) = \frac{\text{Number of pegs per plant}}{\text{Number of flowers per plant}} \times 100$$

At the 20th day after the treatments, top stratum of the biggest leaflet from the second compound leaves was chosen randomly to measure the photosynthetic parameters (*P*n, *G*s, *C*i and *T*r) with a LI-6400 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA) in a clear morning from 10:00 am to 11:30 am under the conditions of light intensity (chamber built in LED) at 1000 μmol m^{-2} s^{-1} and chamber CO_2 concentration at 380 μmol mol^{-1}.

2.3. Extraction, purification and determination of IAA

IAA in plant tissues was analyzed following a protocol modified from Liu et al. (2010). 500 mg of fresh plant materials were frozen in liquid N_2 and ground in a mortar with a pestle, and then extracted by 800 μL of 80% methanol at 4 °C overnight. The sample extraction was centrifuged at 4800 × *g* for 5 min, and the residues were re-extracted with 800 μL of 80% methanol (HPLC grade methanol, Merck, Germany). The supernatants were vacuum freeze dried to dryness at −60 °C, then dissolved in 200 μL of 0.1 mol L^{-1} sodium phosphate buffer (pH 7.8). The aqueous phase was purified though a Waters Sep-Pak C_{18} cartridge (Waters, USA) followed a wash with 200 μL of ddH_2O and then eluted with 1.4 mL of 80% methanol. The eluate with 80% methanol was vacuum freeze dried. The dried extract was dissolved in 40 μL of 50% methanol and used for LC–MS/MS assay in a Agilent HPLC1260-G6460A (LC–MS/MS) system (Agilent, USA). 5 μL of sample was injected into a Acquity UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm). Acetonitrile:water (50:50, v/v) was used as the mobile phase. The flow rate and column temperature were selected to be 0.20 mL min^{-1} and 30 °C. The machine was run with a capillary voltage 3500 V, atomization flow 10 L min^{-1}, cracking voltage 75 V, collision energy 10 V for molecular ions IAA *m*/*z* (174 > 130.1), collision energy 9 V for $^2H_5$-IAA *m*/*z* (179 > 135.2).

2.4. Statistical analysis

All experimental data reported were averages of three replicates and the standard errors (SE) of the means were determined. All statistical analysis were performed with data processing system software DPS version 7.55 (Wang et al., 2010). Microsoft Excel 2003 (Microsoft Corporation, USA) was used to generate graphs.

3. Results

3.1. Auxin and TIBA affect the IAA content in different parts and leaf photosynthetic parameters

Notable changes in the content of IAA took place in different parts of peanut at the 20th day after auxin and TIBA treatments (Table 1). The highest level of IAA content was determined in roots under all the treatments. Spraying 10 μM auxin resulted in

Table 1
Effects of IAA and TIBA on the contents of IAA in different parts.

Treatments	IAA Content in peanut (ng g^{-1} FW)				
	Root	Stem	Leaf	Flower	Peg
Auxin	16.01 ± 0.19a	9.35 ± 0.27a	9.15 ± 0.17a	3.56 ± 0.15a	21.01 ± 1.39a
TIBA	9.19 ± 0.39c	6.21 ± 0.16b	6.38 ± 0.17b	1.21 ± 0.09c	4.39 ± 0.18c
Control	12.56 ± 0.21b	2.73 ± 0.15c	3.26 ± 0.13c	2.28 ± 0.15b	10.25 ± 0.70b

Values are mean ± standard error (SE) of 3 replications. Different small letters within the same short columns indicate significant differences between treatments according to Duncan's multiple range test at $P < 0.05$ level.