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Relationship between Pod Development and Endogenous Cytokinin Content of the Floral Organ in Peanut

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ABSTRACT: To find out the relationship between pod development and cytokinin contents during reproductive stage of peanut, the cytokinin contents, *trans*-zeatin riboside (*t*-ZR) and dihydrozeatin riboside (diZR), were investigated at 0, 7, 14, 21 and 28 days after flowering (DAF). The amounts of *t*-ZR and diZR in cotyledon and first branch among primary branches were 3,448 pmol/g (FW) and 4,824 p/g (FW), respectively, which were higher than those of other branches. The *t*-ZR and diZR contents of lower parts on the branch from cotyledon node at 7 DAF were 579 pmol/g (FW) and 2,028 pmol/g (FW), respectively, which were higher than those of upper parts. The cytokinin contents of reproductive organs as flowering progressed were increased at 0 and 14 DAF on branch and position of node. The cytokinin contents of upper part with pruning the lower part on the branch from cotyledon node were high 112~337% at 7 DAF and 14 DAF compared with those of the control. In case of remove the upper part of the first internode on main axis, *t*-ZR contents was 4.7 times higher than diZR contents at 7 DAF. The pod setting rate of flower and position on the branch from cotyledon node was closely related to the cytokinin contents during floral reproductive stage.

Keywords : Peanut, Cytokinin, *trans*-Zeatin riboside (*t*-ZR), Dihydrozeatin riboside (diZR)

Cytokinins, a major class of plant hormones, regulate growth and development in higher plants. For example, it promotes cell division, delays leaf senescence, and maintains protein and chlorophyll levels in detached leaves. They also affect plant metabolism and the flow of assimilates and nutrients through the plant (Hall, 1973; Skoog & Armstrong, 1970). Cytokinin-like substances have also been reported in the root nodules of *Vicia faba* and *Alnus glutinosa*. Cytokinins are exported from nodules to the xylem and then move from roots to stem (Henson & Wheeler, 1976, 1977a, b). The biosynthesis of cytokinin happens in meristem or tissue which have growth potential during cereal development, the activity of cytokinin is high at early stage during fruit and seed development (Hall, 1973).

Cytokinins and cytokinin-like activity have been reported to increase or attain maximal levels in the root pressure exudate of several species after floral induction (Beever & Woolhouse, 1974; Davey & Van Staden, 1976, 1978a; Heindl *et al.*, 1982). Heindl *et al.* (1982) had suggested that the flux of ZR and diZR had been involved in the control of reproductive development, since this flux was not directly correlated with specific nodule activity or leaf senescence.

Peanut has special growth habit that it fertilizes above ground and then peg grows to underground, pod forms and enlarges in there (Wynne & Emery, 1974). The ratio of mature pod is very low to 9.2~16.3% because of indeterminate flowering (Emery *et al.*, 1981; Lee & Park, 1984a, b). Some researchers studied to improve the ratio of setting and uniformity degree of pod by the treatment of growth regulators such as succinic acid 2,2-dimethyl hydrazide (SADH), 2,3,5-triiodobenzoic acid (TIBA) and choline salt maleic hydrazide (C-MH) (Brown *et al.*, 1973; Reddy *et al.*, 1989; Lee & Kim, 1989). The ratio of pod setting is high on the low position of cotyledon and the first branch, but is low on the high position of the other branches (Carlson *et al.*, 1987). This is closely related with the difference of pod setting ratio between lower and upper parts on flower cluster according to cytokinin contents on every position in assimilation product transfer of flower from leaf of plant, flow of flower cluster.

This study was carried out to investigate the relationship between pod development and endogenous cytokinin contents according to analysis *t*-ZR and diZR contents during reproductive stage in peanut.

MATERIALS AND METHODS

Peanut cultivar Nakateyutaka with 6~7 leaves was trans-

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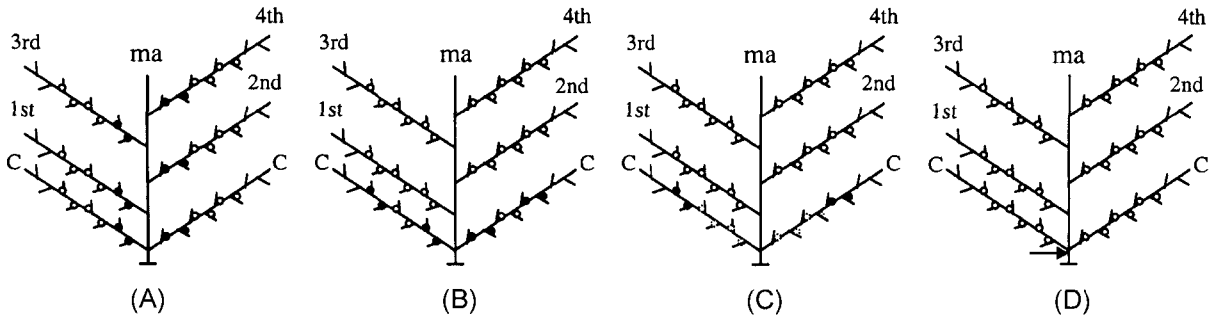

Fig. 1. Picking up positions from branches in peanut. (A); the lower parts of five branches on main axis, (B); the lower and upper part on the branch from cotyledon node, (C); the upper part on the branch from cotyledon node after cut flowers on lower node, (D); cutting point of the first internode on main axis. ma; main axis, C; cotyledon branch, 1st~4th; branches, ; flowers, ● ; picking up flowers, ○ ; removing flowers, → ; cutting point.

planted one plant on pot (40×50×30 cm) filled up with silt loam and cultivated in the greenhouse. Temperature was controlled at 25±1°C and photoperiod was natural light. Soil moisture was maintained to prevent water stress irrigating by automatic machine.

Samples were picked up the flower and flower style to investigate the cytokinin contents, *trans*-zeatin riboside (*t*-ZR) and dihydrozeatin riboside (diZR) at flowering day, which were the five lower parts of branches on cotyledon node and from the first to forth (Fig. 1A). In branch from cotyledon node, samples were picked up on the upper (including the fifth and sixth node) and lower (the first and second node) part (Fig. 1B) according to picking up time at 0, 7, 14, 21 and 28 days after flowering (DAF). It was picked up on the upper (the fifth and sixth node) part after cut flowers on the lower node (Fig. 1C). Samples were a flowers and flower stalks at flowering day but pegs and immature pods since 7 DAF.

Picked samples were stored in -80°C freezer after cutting and freezing by liquid nitrogen. These were homogenized using physcotron homogenizer at cooling by ice and then were separated using micro-centrifugal cooling system (Kubota KR/702), that were stored in 0~5°C refrigerator after equivalence to 25 μl adding 80% methanol. 5 μl of those was concentrated using concentrator VC-960 and then was diluted to 1 μl with *tris*-buffer.

It was gathered bleeding fluids from cutting point of the first internode on main axis (Fig. 1D), which was shut tightly in vinyl pocket after picking to restrain evaporation. Bleeding fluid was measured rapidly after absorption on cotton in vinyl pocket of dark condition at 09:00~10:00 am during two hours Amounts of *t*-ZR and diZR were analyzed using Plant Growth Regulator Detection Kit (Sigma Co.).

The ratio of gynophore formation and ratio of pod setting from cotyledon node branch were investigated according to cut of upper part on the branch from cotyledon node.

RESULTS

Cytokinin contents according to setting position of flower on primary branch

In the result of *t*-ZR and diZR contents analyzed from floral instrument, the first branch contained the highest *t*-ZR contents of 1,299 pmol/g (FW), and the branch from cotyledon node, the third, and the second branches in order (Fig. 2). Contents of diZR were 2.7~6.3 times higher than those of *t*-ZR. Among 5 branches, the first branch contained diZR contents of 3,525 pmol/g (FW) but the others contained similar levels to *t*-ZR contents. Total cytokinin contents (sum of *t*-ZR and diZR) was 4,824 pmol/g (FW) on the first branch, 3,448 pmol/g (FW) on the branch from cotyledon node and 2,700 pmol/g (FW) on the second branch.

Cytokinin contents according to setting position of flower and picking time

Cytokinin contents between lower (the first and the second nodes) and upper (the forth and the sixth nodes) part

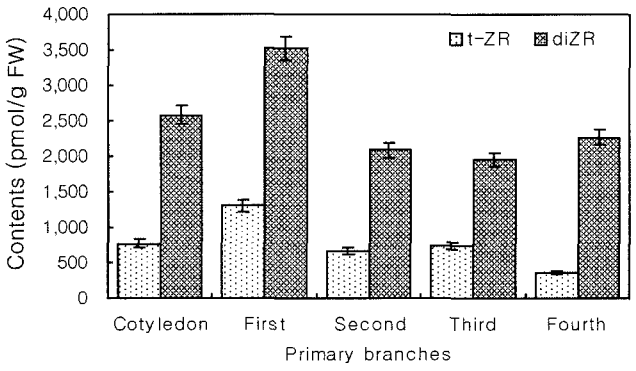

Fig. 2. Cytokinin contents of different primary branches in peanut. Vertical bars are SE.

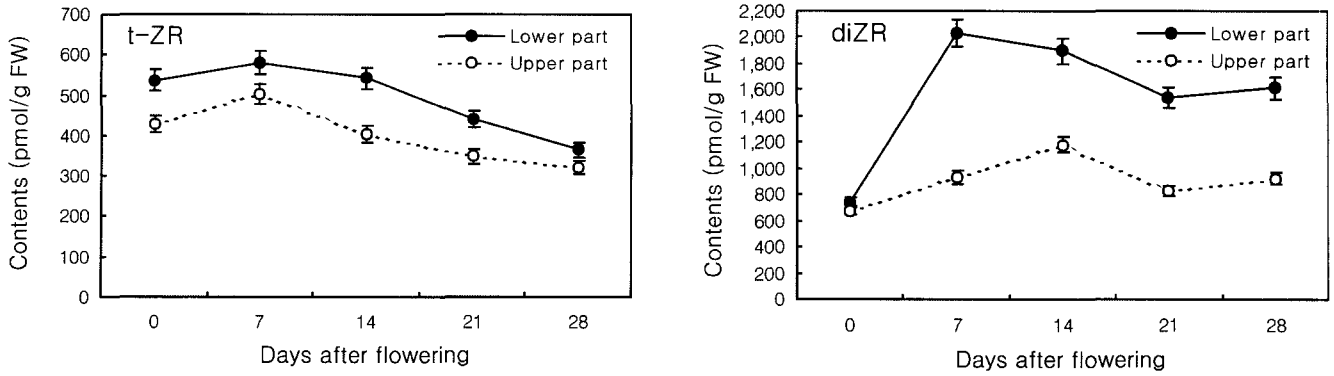

Fig. 3. Cytokinin contents of both flower positions according to picking time on the branch from cotyledon node during reproductive sta ge in peanut. Vertical bars are SE.

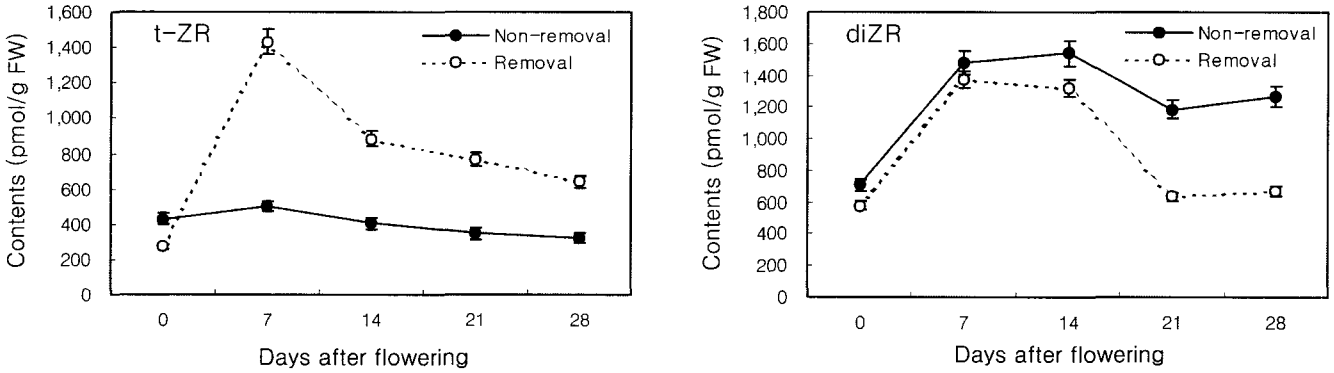

Fig. 4. Cytokinin contents of upper part at removing lower part on the branch from cotyledon node during reproductive stage. Vertical b ars are SE.

according to picking time were showed in Fig. 3. *t*-ZR contents in lower part was 579 pmol/g (FW) at 7 DAF, similar to 537 pmol/g (FW) and 543 pmol/g (FW) at the flowering day (0 DAF) and 14 DAF but low by 440365 pmol/g (FW) at 21 DAF and 28 DAF, respectively. diZR contents in lower part was 2,028 pmol/g (FW) at W7 DAF and was similar to t-ZR contents except on the flowering day, diZR contents 1.4~4.4 times higher than *t*-ZR contents. *t*-ZR and diZR contents in upper part were similar tendency to those of picking time but total *t*-ZR contents was less 12~20%, and diZR contents was less 9~54%.

Cytokinin contents in upper part according to picking time by removing the flower on lower part

In case of removing the flower on lower part (from the first to forth nodes), the changes of cytokinin contents according to picking time were showed in Fig. 4. *t*-ZR contents according to picking time was 2731,431 p/g(FW) in removal and 322502 pmol/g (FW) in non-removal. It was 62% higher as 799 pmol/g (FW) in removal than non-removal, in picking time, hence, it was the highest 1,431 pmol/g (FW) at 7 DAF. diZR contents according to picking time were 580~1,376

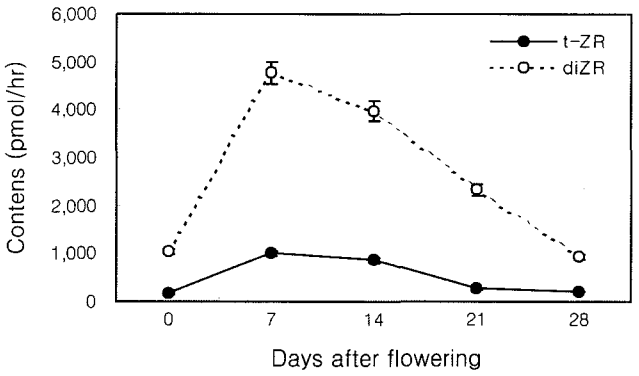

Fig. 5. Cytokinin contents in bleeding fluid from cutting part of main stem during reproductive stage in peanut. Vertical bars are SE.

pmol/g (FW) in removal and 709~1,541 pmol/g (FW) in non-removal. It was the most as 1,480 pmol/g (FW) at 7 DAF in non-removal than removal, however its contents was decreased as picking time was delayed.

Cytokinin content in bleeding fluid by removing the main axis

In remove the upper part of the first internode on main

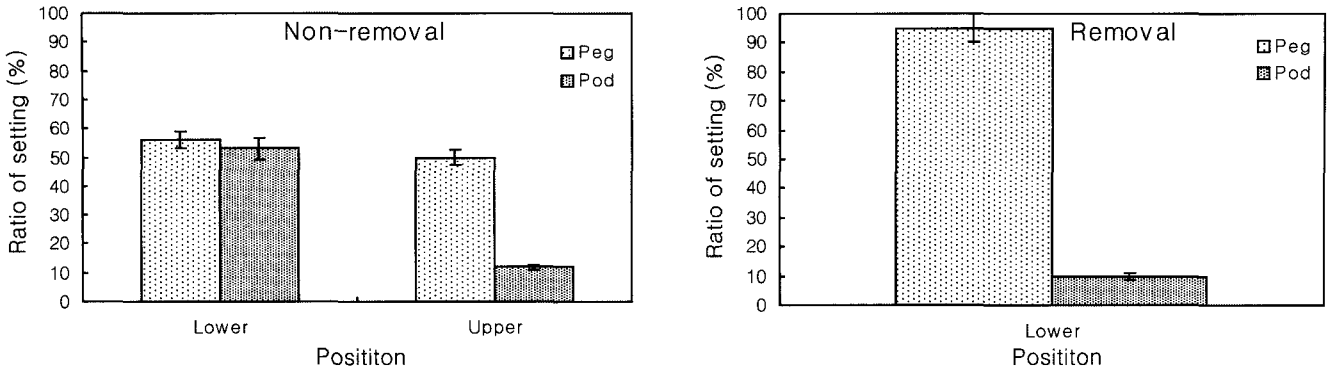

Fig. 6. The ratio of peg formation and pod setting according to different flower position on the branch from cotyledon node in peanut. Vertical bars are SE.

axis, cytokinin contents according to picking time were showed in Fig. 5. *t*-ZR contents was 4,762 pmol/hr at 7 DAF and diZR contents was 999 pmol/hr. In all picking time, diZR contents was higher 725~3,763 pmol/hr than *t*-ZR contents.

Pod setting and peg ratio at different flower position on the branch from cotyledon node

In case of non-removal, the ratio of peg formation was 56% on lower part and 53% on upper part, it was 3% higher than those of lower part. In case of removal, however, it was 95% on upper part that was 42% higher than those of non-removal on upper part. The ratio of pod setting, however, was 50% and 12% on lower and upper part in case of non-removal, respectively, that was 10% higher than on upper part in case of removal (Fig. 6). This looks that pegs on lower part mostly developed to pod in case of non-remove, also it is previewing that the number of flower was increased by way of peg. Therefore it is show that these are affinity between cytokinin contents on upper part and pod setting.

DISCUSSION

Our results demonstrate the relationship between pod development and endogenous cytokinin contents during reproductive stage in peanut. Cytokinin contents of flower were high in the first branch, the branch from cotyledon node and the second branch in order according to flowering position in peanut. According to picking time, it was much at 7 DAF, at flowering day and at 14 DAF in order even though branch and flower were removed or not. This is similar to pod setting every branch in peanut cultivation, and shows that cytokinin content in flower and pod setting is closely related.

Sitton *et al.* (1967) and Wareing *et al.* (1968) have proposed that a decrease in cytokinin flux to the leaves after flowering is responsible, in part, for the leaf senescence, which accompanies fruiting in monocarpic species. The rapid decline in cytokinin flux during pod formation and the continued low levels during seed development that we observed in peanut are consistent with this interpretation. Davey & Van Staden (1978b) detected cytokinin-like activity in the sap (presumably xylem and phloem) entering white lupin fruits. Further, cytokinin-like activity was also present in developing seeds and pod walls. Although it is well known that cytokinins promote cell division in tissue cultures, Davey & Van Staden (1978b) suggested that the cytokinins in these tissues affected seed growth by attracting nutrients to the developing fruits.

Cytokinin activity is high during early stages of fruit and seed development (Hall, 1973). Thus, they offer a possible means to enhance reproduction of peanuts. Ketring & Schubert (1981) have been treated on ground growing peanut with Cytex[3], which is a water-soluble plant (algae) extract containing about 100 ppm kinetin equivalents by bioassay. They, however, have been unable to obtain consistent and significant effects on reproduction of peanut with foliar Cytex sprays. Thus, it is presumed that endogenous cytokinin or cytokinin-like substances are more effective on pod development in peanut than exogenous substances. Hence, aqueous extracts of marine algae contain substance with cytokinin activity (Brain *et al.*, 1973), such extracts increased the seedling emergence rate of fescue (Button & Noyes, 1964), and foliar sprays increased potato yield (Blunden & Wildgoose, 1977).

In summary, we have shown that the contents of ZR and diZR in the transpiration stream change significantly during peanut development. These cytokinins may be involved in the control of reproductive development. It seems that cytokinins serve some function in regulating the coordinated development of the plant, but the precise nature of this regulation is not clear.

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