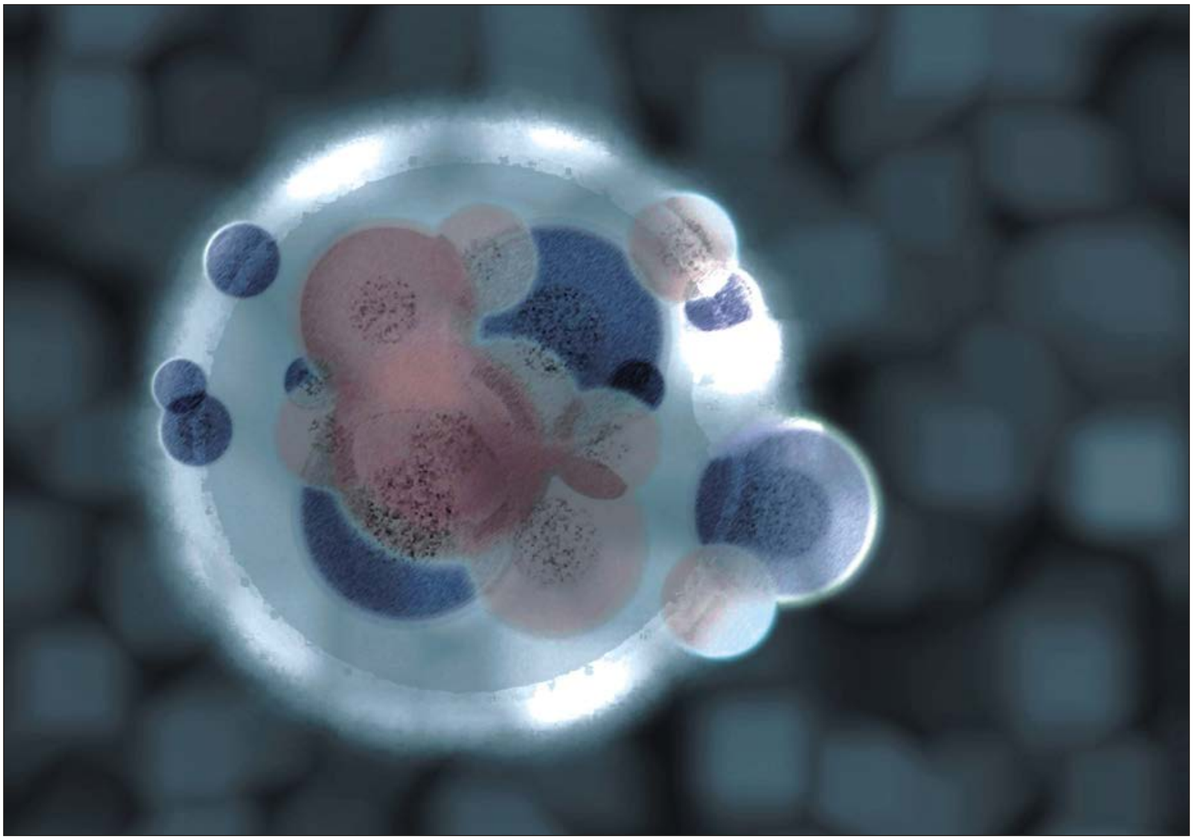

Journal of Biological Sciences

ISSN 1727-3048

ANSInet
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Journal of Biological Sciences 12 (5): 308-314, 2012
ISSN 1727-3048 / DOI: 10.3923/jbs.2012.308.314

Effect of Low Phosphorus Stress on Endogenous Hormone Levels of Different Maize Genotypes in Seedling Stage

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Abstract: The study was aimed at revealing the effect of low phosphorus stress on endogenous hormone levels of maize lines and displaying the different regulars of hormone change under phosphorus starvation between high P-efficient lines and low P-efficient lines, as well as providing the theoretical foundation for identifying high P-efficient lines at the seedling stage. In the present research, two high P-efficient inbred lines of maize 178 and 129, two low P-efficient ones 9782 and 202, with the F1 generation of 178 and 9782 were conducted to assay for the dynamic change of endogenous hormones in immature leaves and roots under low-phosphorus treatment by the method of solution culture, sand culture and field cultivation. As a result, all of the hormones except ABA were observed more abundant in the high P-efficient lines and F1 hybrid than the low P-efficient lines. The contents of zeatin in leaves and roots decreased significantly by the stress of phosphorus starvation under all of the environments, contrarily the contents of gibberellic acid (GA3), Indol-3-acetic acid (IAA) and Abscisic Acid (ABA) increased after suffering the low-P stress. In addition, the change ranges of GA3 and IAA were greater in high P-efficient lines 178, 129 and the F1 hybrid than in low P-efficient 9782 line and 202 line, whereas it is the opposite case for Zeatin (ZT) and Abscisic Acid (ABA) contents. However, no significant difference of every hormone change was observed between the two high P-efficient inbred lines as well as the two low P-efficient ones. Which indicated that the distinction of the hormone sensibilities to phosphorus starvation were just specifically present among the lines with different P-availability and can be considered as an important reference basis for identifying phosphorus efficiency of maize lines. Each of the hormones in the F1 hybrid always displayed the highest or the lowest values among all the lines, indicating the heterobeltiosis of F1 generation in response to low starvation. So the method of cross breeding can be considered to applied to breeding higher P-efficient lines of maize.

Key words: Maize, low-phosphorus stress, phosphorus efficiency, endogenous hormones, multiple environments, correlation analysis

INTRODUCTION

Phosphorus (P), an essential macronutrient for plant growth, provides indispensable substance foundation to agricultural production (Nagarajan *et al*., 2011). As a kind of non-renewable resources, phosphorus in the earth was suggested to supply the demand for only 50 to 400 years, due to overexploitation by human beings (Wang and Li, 1998). The shortage of phosphorus will become a worldwide resource crisis in the near future. At the present time currently, P deficiency has been a serious problem in 43% of the world's total cultivated land (Liu and Li, 1994). Even worse is that no mineral element is found to be able to displace phosphorus in agricultural production so far. So illustrating the mechanism of phosphorus utilization and creating more high P-efficient plants is a necessary strategy for relieving the pressure from P shortage (Rausch and Bucher, 2002).

There is significant difference on phosphorus efficiency among various plant species, as well as different genotypes from the same species based on genetic variation (Buso and Bliss, 1988). Smith (1934) screened 24 inbred lines and 23 single crosses of maize to determine the relative amount of growth with various limitations of phosphorus. The results showed there were significantly differential responses among them when grown on a medium in low phosphorus (Smith, 1934). Similar evidence was found in the research on wheat, rice, sorghum and other species (Gabelman and Loughman, 1987). These findings provide important materials and evidence for studying physiologic and genetic mechanism of phosphorus efficiency. As well

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known to us, endogenous hormones participate in many of physiologic process in plant growth and act as signal substances when plants encounter environmental stress (Srivastava, 2002). The effect of different phosphorus concentrations on growth and physiology characteristics of barley have been identified which revealed that the P-starvation led to increased IAA and GA3 content and decreased IPA content in roots and leaves. However no significant change was observed for ABA content by low-P stress (Liu and Wang, 2003). Similar conclusion has been also demonstrated in the research of cotton (Wang *et al*., 2008). It is suggested that the effects of P stress on the growth of barley and cotton may be associated with the changes of endogenous hormones in the plants.

But the dynamic change of endogenous hormones in maize under low-P stress is still unclear. And it is unknown whether different responses of endogenous hormones are present among various P-efficiency genotypes. In the research, two high P-efficient inbred lines and two low P-efficient ones, with their cross F1 were conducted to assay for the dynamic change of endogenous hormones in immature leaves and roots under low-phosphorus stress under the condition of solution culture, sand culture and field cultivation. It will help to understand the mechanism on hormone response to low P-efficient in maize and afford us an advantageous and convenient method for identifying high P-efficient lines in early growth period of maize.

MATERIALS AND METHODS

The study was performed in Maize Research Institute of Sichuan Agricultural University, Ya'an city of China. The time duration of the research was about eight months from March to October in 2011.

Solution culture: The seeds of maize (*Zea mays* L.) were sown on filter paper saturated with distilled water and incubated at 26°C in the dark. Three days later, seedlings selected for uniform growth were transplanted into an aerated complete nutrient solution (Table 1) and kept for 3 d in a growth chamber with a photoperiod of 14 h light/10 h dark at 26°C and relative humidity of 70%. Then these seedlings were randomly divided into two groups (each group includes three replicates), one of which was transferred to a fresh solution same to the above (normal-P level, NP), the other was transplanted into an incomplete solution with only 1 μM phosphorus (low-P level, LP), in which the other compositions were identical to the former.

Sand culture: Twelve plants were grown in two rows for every of the above line, with 15 cm row distance, 6.7 cm plant distance. Which was designed for two replicates and each one was randomly ordered. There was only the difference of monoammonium phosphate on fertilizer for LP and NP, with 8 g $(NH_4)_2HPO_4$ to NP and no to LP in each hill. The other fertilizer was used on normal levels.

Table 1: Gradient elution program

Time (min)	Methanol (%)	0.6% Acetic acid (%)
0	4	96
10	50	50
30	50	50

Field cultivation: Maize lines were planted in Xiali town of Ya'an City, where the content of efficient phosphorus in soil is lower than 10 mg kg^{-1}. Twelve plants were grown in a row for every of the above lines, with 50 cm row distance, 33 cm plant distance. Three replicates were designed with randomly order. Sixty kilograms of phosphorus was fertilized in per square hectometer field for NP, no P for LP. Field management and the other fertilizer were in accordance with normal levels.

At six-leaf stage, about 2.0 g roots and leaves were respectively conducted to screen for endogenous hormone content using HPLC.

Determining of endogenous hormone content: About 2.0 g tissue was put into a cooled mortar, added with 15 mL 80% methanol and grinded into homogenate, kept still at 4°C for 12 h. Then the homogenate was centrifugalized at 5000 rpm for 5 min and the supernatant fluid was saved at 4°C. The remainder was conducted to the above steps for three times. All of the supernatant was mixed and concentrated to 10 mL volume at 35°C, appended with 0.1 g Polyvinylpyrolidone (PVPP) to remove pigments and phenols. After being mixed for 1 h and centrifugalized at 4°C at 10000 rpm for 15 min, the crude extract of endogenous hormone was collected from the supernatant. Every 30 mL extracting solution was transferred into a culture dish with 9 cm diameter and dried by a freeze drier. Each dish was dissolved by methanol for three times and the solution was transferred to a 10 mL volumetric flask with constant volume. After filtered by 0.22 μm membrane, the solution was subjected to determine the content of endogenous hormone.

The content of endogenous hormone was determined using high performance liquid chromatograph Agilent 1 100 LC, with chromatographic column Hypersil ODSC$_{18}$. The mixture of methanol and acetic acid were used as moving phase and gradient elution program was showed in Table 1. The other conditions were as follows: column temperature of 35°C, sample size of 10 μL, flow speed of 1 mL min^{-1} and 254 nm wavelength. The External Standard Method was applied to calculate hormone content.

RESULTS

Decreased zeatin content under low-P stress at seedling: For every of the culture conditions, zeatin in roots and leaves was both decreased under low-P stress but the reduction range was larger in roots than leaves concerning all of the maize lines (Fig. 1). Which indicated that zeatin in roots is more sensitive than in leaves to P starvation stress. There were also significant difference of Zeatin content between high P-efficient inbred lines (HPELs) and low P-efficient inbred lines (LPELs) under normal condition. It was embodied in higher zeatin level in HPELs and lower level in LPELs. For example, under the condition of normal P-level solution culture, Zeatin in the leaves of 178 and 129 L were, respectively 165 ng g^{-1} FW and 185 ng g^{-1} FW but only 125 ng g^{-1} FW and 110 ng g^{-1} FW in line 9782 and 202. Similar data was found in the roots of the above lines. Interestingly, zeatin contents both in leaves and roots fell to lower levels in LPELs than in HPELs and F1 without exception. It suggested that keeping zeatin on a relatively high level would be a co-characteristic for HPELs. Additionally, zeatin contents appeared more steady under field cultivation than the other cultures, when encountering low-P stress.

A little increased GA_3 in HPELs under P starvation condition: Under the normal condition, GA_3 in the three HPELs was far higher than that in all of the LPELs and the F1 hybrid possessed the largest GA_3 content among the lines (Fig. 2). Compared to zeatin, GA_3 content in all the lines changed more faintly after low-P stress under the three conditions. For instance, there was an up-regulation by less than 2% of GA_3 content in line 178's root and leaf on low-P level. But significant difference of change range was still found between LPELs and HPELs. As for HPELs, GA_3 contents in roots and leaves rose significantly with no exception, whereas no distinct increase was observed for LPELs. However, there was no difference of increase range among the different cultures, also between roots and leaves. It is suggested that failing to promote signal transduction by GA_3 is an important reason for the impaired growth and development of LPELs under P-limited circumstances. As for HPELs, a little increased GA_3 may transmit a signal of P starvation to plants, promoting the timely regulation of root architecture to response to P deficiency.

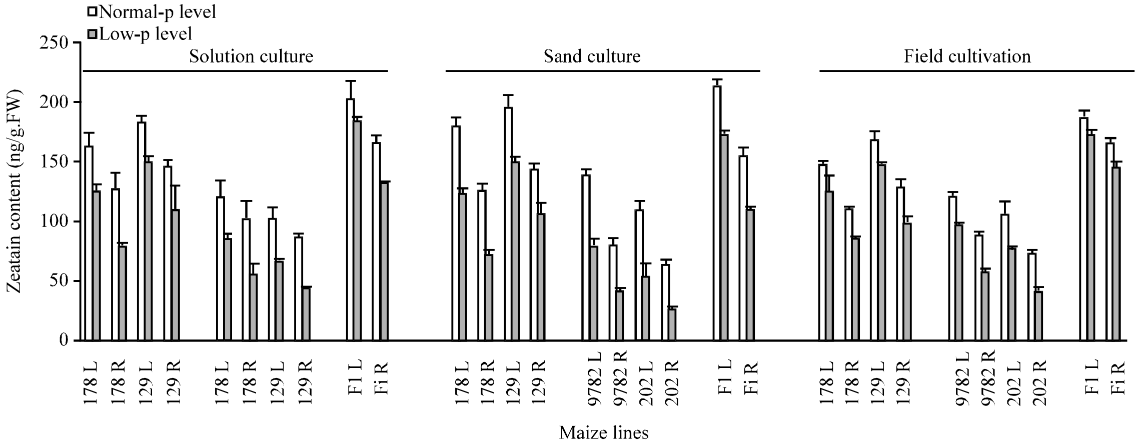

Fig. 1: Zeatin content change in different culture conditions with low-P stress, L: Leaf, R: Root

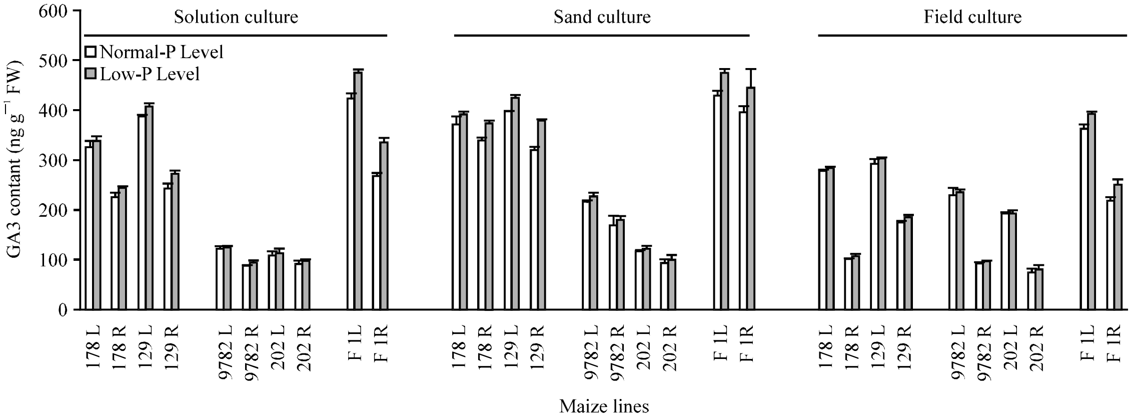

Fig. 2: GA3 content change in different culture conditions with low-P stress, L: Leaf, R: Root

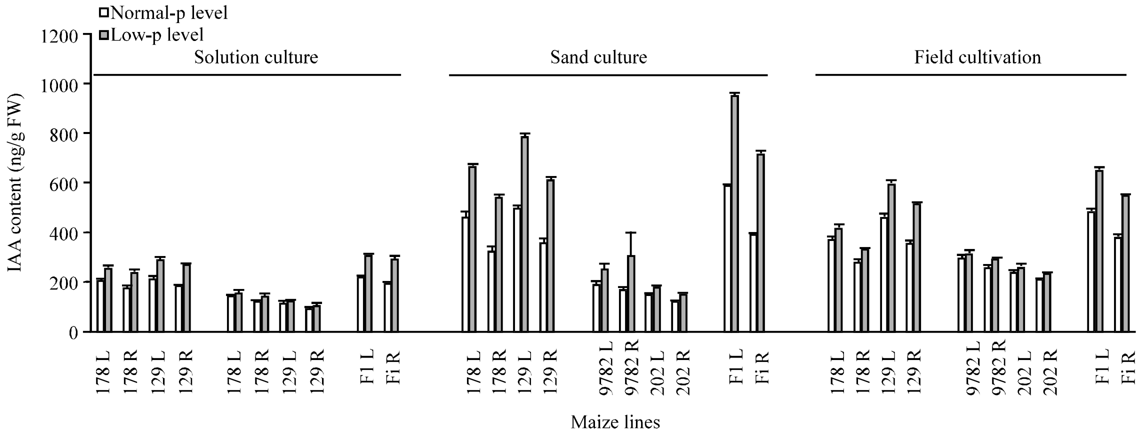

Fig. 3: IAA content change in different culture conditions with low-P stress, L: Leaf, R: Root

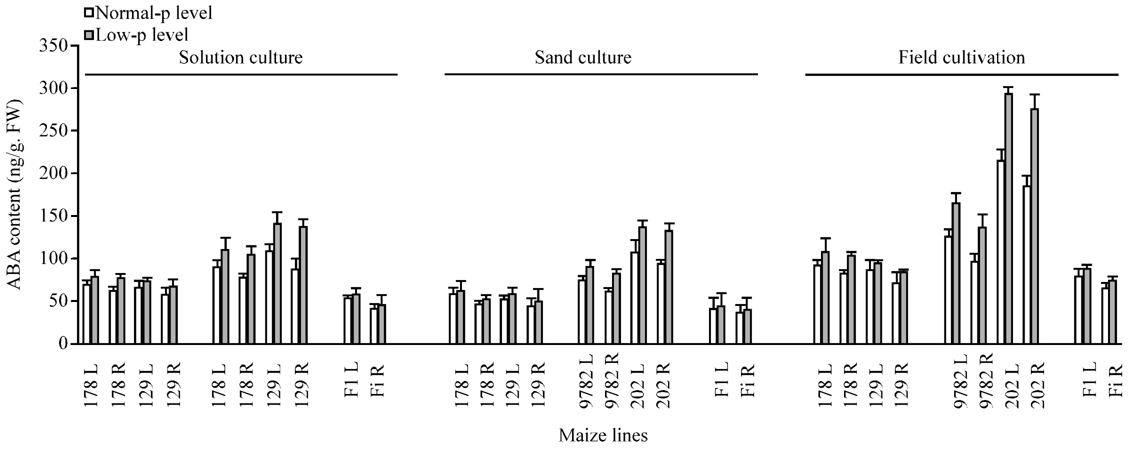

Fig. 4: ABA content change in different culture conditions with low-P stress, L: Leaf, R: Root

Increase of IAA between LPELs and HPELs under P-deficient stress: Similar to GA_3, there was also great disparity on IAA content among LPELs, HPELs and F1 hybrid under normal condition (Fig. 3). The content in F1 was the largest, then followed by HPELs and the smallest in LPELs. Additionally, IAA was always more abundant in leaves than in roots for a given line. Under low-P stress, increasing degree of IAA contents in F1 and HPELs were far higher than those in LPELs. Concerning various culture conditions, there was very similar increasing trend of IAA content between solution and sand culture. But the determined IAA content under the condition of solution culture was much less than ones in sand culture and field cultivation. Whereas, IAA of the plants cultivated in fields is the most insensitive to low-P stress among the three culture condition. The probable cause was that the actual phosphorus level in field circumstance was higher than what in solution and sand condition because of the existence of the inherent phosphorus in soil.

Increased ABA in LPELs than HPELs under low-P stress: There was also remarkable difference on ABA content among LPELs, HPELs and F1 hybrid in normal-P level which was embodied in the lowest in F1 hybrid, the second in HPELs, the highest in LPELs (Fig. 4). After suffering low-P stress, ABA increasing range in LPELs was larger than in HPELs and F1 hybrid for all of the culture conditions. It is suggested that ABA in LPELs is more sensitive to low-P stress. On the other hand, ABA is always more abundant in leaves than in roots for a given line at the same phosphorus levels. But the increasing extents in roots were higher than in leaves for every of the maize lines. Which revealed that ABA in maize roots is more susceptible to P starvation than the one in leaves. Moreover, the values of ABA content determined under field circumstance were unanimously larger than both solution and sand culture. Whereas, the change tendency of them is consistent among the three culture environments.

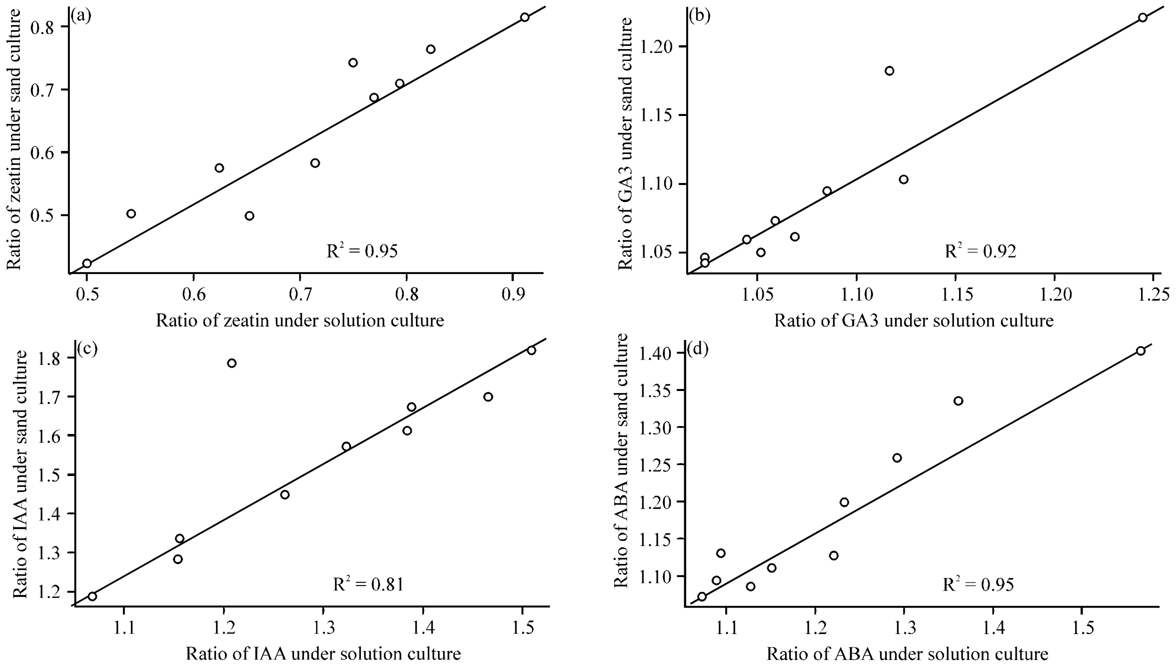

Fig. 5(a-d): Correlations of hormone (a) Zeatin, (b) GA3, (c) IAA and (d) ABA of low-P to normal-P ratios between solution and sand culture

Correlations of hormone ratios of LP to NP between solution culture and sand culture: To validate the correctness of determined hormone contents, all the hormone ratios of low-P level to normal-P level was submitted to the software of correlation analysis (https://www.wessa.net/rwasp_correlation.wasp?outtype=Browser%20Black/White#output) (Shen *et al.*, 2012). As shown in Fig. 5, a strong correlation (all Pearson's correlation more than 0.90) was revealed between the data from solution culture and those from sand culture (a, b, d) except for the IAA ratios (Fig. 5c, R^2 = 0.81) which indicated a good concordance of both the methods. The lower correlation coefficient of IAA ratios may suggest IAA content is more susceptible to environment.

DISCUSSION

Phosphorus is a constituent of important organic compounds. It is commonly assumed that the effects of varying levels of phosphorus on plant growth can be directly related to its ability for basic anabolic processes such as protein, nucleic acid and membrane synthesis (EI-D *et al.*, 1979). To response to such variations, plants have evolved complex sensing and signaling mechanisms that allow them to monitor the external and internal concentration of phosphorus. Recent evidences have shown that hormones participate in the control of these regulatory networks (Rubio *et al.*, 2009).

Zeatin, a sort of cytokinins which promote cell division or cytokinesis in plant roots and shoots and were ever proved to play opposite roles in plant lateral root formation (Lohar *et al.*, 2004). In our study, phosphorus deficiencies resulted in reduced zeatin which is in agreement with those of the earlier studies of Kulaeva (1962), Wagner and Michael (1971) and EI-D *et al.* (1979), who all found reduced cytokinin levels were associated with mineral deficiency. Moreover, the increased lateral root number and length was observed under low-P stress. Based on the above findings, it is proposed that cytokinin production may be related to P nutrition. Phosphorus deficiency in roots leads to decrease of zeatin in roots, resulting in increased maize lateral roots consequently. On the other hand, zeatin decrease ranges in all the HPELs were smaller than those in LPELs which suggests P availability is also able to change the zeatin level. When encountering low-P stress, HPELs improved P uptake to increase available phosphorus, minimizing the reduction range of zeatin in roots.

GA_3 is a simple gibberellin, a tetracyclic diterpene acid promoting growth and elongation of cells. Biosynthesis of GA is regulated by these genes CPS, KS, KO, GA3ox1 and GA20ox1 (Devaiah *et al.*, 2009). Previous research revealed that GA_3 could promote plant root growth by influencing root cell elongation (Jiang *et al.*, 2007). The role of GA has been proven to be implicated in

P starvation response. The cross-talk between P starvation stress and GA biosynthesis via transcript factor MYB62 was also demonstrated in *Arabidopsis*. P starvation resulted in down-regulation of MYB62 and subsequent up-regulation of the above genes required for GA biosynthesis consequently. Which, lead to vital modifications in plants required for survival under P deficiency (Devaiah *et al*., 2009). In the present research, a little increased GA_3 content was observed in both HPELs and LPELs. The results are in accordance with the findings on barley and cotton study (Liu and Wang, 2003; Wang *et al*., 2008), confirming the cross-talk between low-P stress and GA_3 in maize. Additionally, GA_3 increase range in HPELs was higher than in LPELs under low-P stress, indicating that the former was superior to the latter on regulating root architecture and promote primary roots growth after suffering P deficiency.

IAA is the most important member of auxin family and essential for plant body development, having a cardinal role in coordination of many growth and behavioral processes in the plant's life cycle (Teale *et al*., 2006). Although the IAA biosynthetic pathway in plants has not yet been determined with certainty, a large body of evidence has accumulated showing that plants convert Trp to IAA via several parallel pathways with these enzymes, YUCCA and $CYP_{79}/CYP_{79}B_3$ involved (Yamamoto *et al*., 2007). Miura *et al*. (2011) proposed hyperaccumulation of auxin facilitates low-P induced root of *Arabidopsis* architecture remodeling (Miura *et al*., 2011). Similar data was observed on *Lupinus albus* under phosphorus deficiency (Shen *et al*., 2008). Which are consistent with our research findings. It is suggested that P starvation may cause the change of the enzymes such as YUCCA and $CYP_{79}/CYP_{79}B_3$ and lead to increase of IAA in root consequently. The difference of IAA change range between HPELs and LPELs may account for the distinction of P availability between them.

ABA functions in many plant developmental processes. ABA-mediated signalling plays an important part in plant responses to environmental stresses, such as drought, chilling, high salinity, phosphate deficiency and nitrate enrichment (Zhu, 2002). An increased ABA content was considered to be beneficial for plants under environmental stress as a result of ABA-induced changes at the cellular and whole-plant levels (Xiong and Zhu, 2003). ABA promotes the closure of stomata to minimize transpirational water loss. It also mitigates stress damage through the activation of many stress-responsive genes that encode enzymes for the biosynthesis of compatible osmolytes and LEA-like proteins which collectively increase plant stress tolerance (Hasegawa *et al*., 2000; Bray, 2002; Sharp and LeNoble, 2002). These evidences support our observation in the study. The regulation process of ABA biosynthesis has been illustrated. In the pathway, phosphoprotein cascade is a series of important P-proteins affecting ABA biosynthesis (Xiong and Zhu, 2003). It is proposed that low-P stress may lead to, first of all, the change of the phosphoprotein cascade, then result in ABA increase.

Three different medium cultures were adopted in the study, receiving accordant results about hormone change ranges, verifying the correctness on experimental data.

CONCLUSION

By the research distinct change ranges of the endogenous hormones were observed between high P-efficient inbred lines and low P-efficient ones of maize when they encountered the stress of P starvation. However, no significant difference of each hormone change was detected between the two high P-efficient inbred lines as well as the two low P-efficient ones. Which indicated that the distinction of the hormone sensibilities to phosphorus starvation were just specifically present among the lines with different P-availability and can be considered as an important reference basis for identifying phosphorus efficiency of maize lines at the seedling stage. Each of the hormones in the F1 hybrid always displayed the highest or the lowest values among all the lines, indicating the heterobeltiosis of F1 generation in response to low starvation. So the method of cross breeding can be considered to applied to breeding higher P-efficient lines of maize.

ACKNOWLEDGMENT

This research was supported by the National Natural Science Foundation of China (grant No. 30971796 and 30900901).

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