

Applications of signal transduction and xerophytophysiology by exposing hypocotyls in organic peanut production

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Key words: AnM cultivation, anthocyanin, *Gdi-15* gene, peanut (*Arachis hypogaea*), signal transduction, xerophytophysiology

Abstract

The AnM practices in peanut production included three steps. A, n and M, showed the section-cross of the ridge at different peanut growth stages. First, seeds were sown deeper than usual to induce extra-elongation of hypocotyl. Then the ridge cross-section looked like "A". The second, the hypocotyls elongated more than usual were exposed to light and dry air by removing the soil away around the young hypocotyls. At this time, the ridge cross-section looked like "n". The third, at the middle growth stages, soils on both sides of ridge were earthed up to welcome the late pegs. Then the ridge cross-section looked like "M". AnM induced osmotic adjustment and improved photosynthesis by a higher leaf turgor. Anthocyanin accumulation was apparent in hypocotyls soon after the exposure started, accompanied by active increase in osmolytes such as sugars. The up-regulation expression of *Gdi-15* gene was found in hypocotyl. The AnM practice was more effective in the soil with compost applied to the surface layer and therefore it is feasible in organic peanut production.

Introduction

The AnM peanut cultivation method is adopted in China (Shen and An 1988). The three letters, A, n, and M, refer to the shapes of the ridge at different stages. The letter "A" shows shape of the cross-section of the ridge after the seeds are sown; the small letter "n" shows the ridge shape at the seedling stage, when the hypocotyls are exposed by removing away the soil around; and the letter "M" shows the ridge shape at the full blossom stage, when soil is earthed up from both sides of the ridge to welcome the pegs. Agronomic advantages showed a yield increase by the AnM method (Shen 1985; Shen and An 1988). However, the related physiological and molecular biological basis for the yield increase have not been well understood since almost thirty years ago. Here, a hypothesis was proposed that AnM method would be a practice of applications of xerophytophysiology in plant production. Exposing hypocotyls to light and dry air might be the key practice to stimulate the young plant to cause drought stress signalling and activate some responsive genes. As reported, *Gdi-15* is a groundnut desiccation-induced gene and would be activated by drought signalling (Gopalakrishna et al. 2001). Therefore, in the present study, one experiment was designed to confirm AnM method with not only the agronomic traits and photosynthetic activities but also the xerophytophysiological regulations such as osmotic adjustment and turgor maintenance. In addition, another experiment was designed to examine the *Gdi-15* gene and the physiological regulations related with its expression.

Material and methods

Experiment 1: Peanut (*Arachis hypogaea* L. cv. Chibahandachi) was grown in organic field with an organic fertilizer (N-52, P-30 and K-20 g kg⁻¹) fermented using oil mill sludge, rice bran and fish meal was applied 200 g m⁻². As shown in Fig. 1, seeds were sown 8 cm deep, the shape of the ridge cross-section was like the letter "A". Two weeks later, the soil around the seedlings was removed away with the hypocotyl exposed to light and dry air and the shape of the cross-section of the ridge was like the letter "n". At full blossom, the soil from both sides of the ridge was earthed up to welcome the pegs and the shape of the cross-section of the ridge was like the letter "M". The ridge was 20 cm high and the space was 30 cm between two plants and 60 cm between two ridges. Compost application to the soil surface layer was taken as the main plot and the AnM cultivation as the sub-plot in a 2x2 factorial split design. Analyses of photosynthesis and osmotic adjustment were according to Xu et al. (2011). Experiment 2: The same cultivar and similar management as in Exp.1 was used. The hypocotyl exposure was started one week after seeds sown, and only "n" stage was involved. The light density to which hypocotyls were exposed was more than 1200 μmol m⁻² s⁻¹ at midday.

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Concentration of anthocyanins was measured by spectrometry. The analysis with real time PCR (Applied Biosystems, USA) for *Gdi-15* was done following the steps in manuals of RNeasy Mini Kit (Qiagen, Tokyo, Japan), PrimeScript® II 1st Strand cDNA Synthesis Kit and SYBR® Premix Ex Taq™ II (TaKaRa, Japan). Primer pairs for *Gdi-15* gene was [forward, 5'-GGTGTCCCATGATTGC-3'; reverse, 5'-GCCTTGGTAGAAGAGCC-3'].

Results

Experiment 1: The shell yield was higher in the AnM plots in both treatments with and without compost application with a positive synergistic interaction between AnM and compost application. Disease of leaf spot was less severe in plots with higher shell yield. Leaf color and photosynthetic capacity were proportional to shell yield. The leaf turgor at the fully turgid status (π_{FT}) was higher due to more active solute accumulation (ΔC_{FT}) in AnM plots. At incipient plasmolysis, both osmotic potential (π_{IP}) and leaf relative water content (ζ_{IP}) were lower in AnM plots, suggesting higher stress tolerance in plants of AnM plots. The symplastic water fraction (ζ_{sym}) was also higher in plants of AnM plots, which might contribute to higher physiological activities. AnM cultivation was more effective in plots with compost applied to the soil surface layer. Experiment 2: The increased expression of *Gdi-15* gene was found in the exposed hypocotyl of the peanut seedling (Table 2). The clear accumulation of anthocyanins also confirmed the enhanced expression of *Gdi-15* gene. In the present experiment, anthocyanin accumulation in response to the hypocotyl exposure is a protective strategy against drought stress. The increased expression of the drought-responsive gene, *Gdi-15*, was found only in the exposed hypocotyl but not in leaves and root, where the stimulation was not directly imposed.

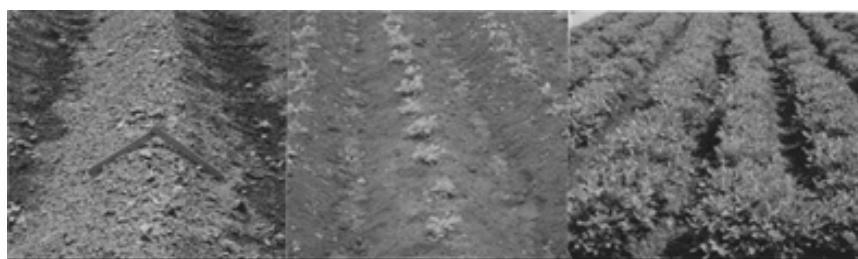
Table 1. Shell yield, disease index, photosynthetic activities and osmotic adjustment parameters under different cultivation practices (Experiment 1).

Compost	AnM	Yield	DI	L color	P_C	P_{FT}	π_{IP}	ζ_{IP}	ζ_{sym}	ΔC_{FT}
No	No	2.73	11.3	48.3	28.1	0.653	-0.984	0.858	0.71	0.0
	Yes	3.06	6.4	50.4	29.2	0.733	-1.161	0.829	0.75	37.1
Yes	No	3.24	8.2	50.2	30.3	0.748	-1.172	0.831	0.74	18.2
	Yes	3.76	4.2	52.1	31.9	0.802	-1.273	0.802	0.77	69.3
Compost		*	*	*	**	**	**	*	*	**
AnM		*	*	*	*	**	**	**	**	**
CxAnM		*	ns	ns	*	*	*	ns	*	**

Shell yield (kg m^{-2}); P_C , photosynthetic capacity ($\mu\text{mol m}^{-2} \text{s}^{-1}$); DI, disease index (%); L color. Leaf color (SPAD); P , π , ζ and C mean turgor potential (MPa), osmotic potential (MPa), leaf relative water content and osmotic concentration (osmol m^{-3}), respectively; Subscripts, FT, IP, and sym, mean those at full turgid status, incipient plasmolysis, symplastic water fraction, respectively. * and ** show significance at $p \geq 0.05$ and $p \geq 0.01$.

Table 2. *Gdi-15* gene transcript levels, anthocyanins concentration (Experiment 2).

Plot	<i>Gdi-15</i> expression				-----In hypocotyls-----	
	Hypocotyl	Root	Cotyledon	Leaf	Anthocyanins ($\text{OD}_{530} \text{g}^{-1}\text{FW}$)	Sugars (g kg^{-1})
CK	3.2	2.62	0.76	11.2	0.14	9.5
AnM	117.3**	1.78 ^{ns}	0.63 ^{ns}	10.4 ^{ns}	7.76**	11.4*



"A" stage "n" stage "M" stage

Fig. 1. The peanut AnM cultivation (left) and color changes (right) after hypocotyl exposing.

Discussion

The AnM cultivation has proved effective in China for many years. The agronomical advantages have been clarified (Shen and An 1988). The present study found that the fraction of the symplasmic water was larger; the osmotic concentration was higher and as a consequence the leaf turgor potential became higher in leaves of hypocotyl exposed peanut plants. The high leaf turgor maintenance is considered as the main one of the mechanisms for the yield increasing effect of the AnM treatment. The key point may be the stimulation by exposing the hypocotyl, whereby a signal can be sent to the internal gene system, where the stress-responsive genes are activated, transcribed and expressed to enhance the physiological activities for protection and stress resistance. *Gdi-15* is one of these drought responsive genes (Gopalakrishna et al. 2001) and related with anthocyanin biosynthesis and many abiotic stress adaptations (Chervin et al. 2009). In the present study, the increased expression of *Gdi-15* gene was found in the exposed hypocotyl of the peanut seedling. The clear accumulation of anthocyanins also confirmed the enhanced expression of *Gdi-15* gene. In plant physiology, foliar anthocyanins are synthesized in response to drought, cold or saline environment and serve as osmotically active solutes to decrease leaf osmotic potential, increase water uptake and maintain leaf turgor potential in addition to functions as UV screen and free-radicals scavenger. The color change, particularly the change in color from white to purple that the anthocyanins might be related, is a typical xerophytophysiological response (Xu 2007). Plant tissues containing anthocyanins are often resistant to drought stress although the drought resistance is not causatively linked to anthocyanin concentration. *Gdi-15* might be considered as the representative of the drought responsive genes (Gopalakrishna et al. 2001). In practice, the hypocotyl is exposed but the root anchors in the sufficiently moist soil without any soil water deficit. Actually, hypocotyl exposure is not a real stress and it is only a stimulus. The increased expression of the drought-responsive gene, *Gdi-15*, was found only in the exposed hypocotyl but not in leaves and root. Nevertheless, as a false stress it successfully induced the increased expression of drought-responsive gene and the consequent xerophytophysiological regulations that might be positive to the crop. This is the key point of practices of xerophytophysiology and signal transduction in plant production, which was proposed by Xu (2007) and also used in other crops, such as mesocotyl exposure for sorghum plants (Xu et al. 2009), clove exposure for garlic plants (Qin et al. 2008a), partial root-zone drying for potato (Xu et al. 2011) crops, and blue light irradiation in canopy for tomato crops (Xu et al. 2012). In most of the practices, the root of plants anchors in moist soils without real water deficit, as in case of the hypocotyl exposure of the peanut seedlings. Results of the present experiment confirmed that, as one of practices of stimulation based on the theory of xerophytophysiology, treatment of hypocotyl exposure in the AnM technique, was effective in inducing enhanced expression of drought responsive gene and the expected consequences of regulations in crop production.

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