論 文 の 内 容 の 要 旨

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論文題目　Biological Fundamentals of AnM Techniques in Peanut Crop Production
（落花生栽培における AnM 法の生物学的基礎）

The AnM technique has been adopted in peanut production and proved effective in improvements of yield and disease resistance. However, the detailed mechanisms for the AnM technique have not been clarified. Therefore, in the present research, several experiments were carried out and biological fundamentals for the growth and yield improvement were examined in addition to confirmation of the agronomical advantages. Moreover, experiments were also conducted to confirm the feasibility of a modified AnM in combination with film mulching and an alternative by transplanting seedlings with extra-elongated hypocotyls, which were suggested easily practiced with mechanization.

The AnM practices included three steps. The three letters, A, n and M, showed the shapes of the section-cross of the three steps at different growth stages of the peanut crop. First, the peanut seeds were sown a little deeper than usual, about 8 cm, in the ridge to induce the extra-elongation of the hypocotyl. When the seeds were sown, the cross-section of the ridge looked like the letter A. The second, the hypocotyls elongated more than usual were exposed to light and dry air by removing the soil away around the young seedlings just after the emergence. At this time, the cross-section looked like the letter “n”. The third, at the middle growth stages, soils on the both sides of ridge were earthed up to welcome the late pegs. At this time, the cross-section of the ridge looked like the letter “M”.

Botanically, the elongation of peanut hypocotyl stops when the cotyledons are sent out of the soil and meet light. However, in most cases, the hypocotyl even stops elongation and leaves the cotyledon node under the soil surface when cotyledons meet light through the soil cracks. Therefore, the flowers on the early two branches from the cotyledon nodes pollinate themselves even under soil surface and produce pods earlier and the early pods compete for nutrition with the young seedlings. Therefore, the key point of “A” step was to lift up the cotyledon nodes out of the soil. At “n” step, soil around the seedling is removed and hypocotyl is exposed to light and dry air. This practice makes early pegs farther from the soil surface and thus the early formation of pods is avoided. Actually, exposing the hypocotyl is the key point of the “n” step and is also the key point of the whole AnM techniques.

In practice the modified AnM, the seeded hole on the surface of plastic films on the ridge is covered with 5-7 cm high of soil mound to induce more elongation of the hypocotyls. The soil on the film is removed after the cotyledon node is sent out of the film surface with the extra-elongated hypocotyl exposed to light and dry air. As an alternative of AnM technique more easily practiced, transplanting of seedlings was tried. Peanut seeds were first sown in seedling packs and placed in dark inside an incubator, where extra-elongation of the hypocotyls was induced. When the hypocotyls elongated to about 5 cm long, seedling packs were moved to a lighting growth chamber. The seedlings were transplanted into the field with the hypocotyls half above the soil surface.

Agronomically, all the three types of AnM practices improved plant biomass production and final shell yield with the disease resistance also increased. The yield increment was 19.2%, 16.7% and 18.9%, respectively, for the basic, modified and alternative AnM techniques. The additive and/or synergistic yield increment reached 71.2% for the modified AnM in combination with film mulching and 33.6% for AnM treatment in addition to seedling transplanting. The bio-degradable black film was better than the transparent film because the former depressed weeds and promoted soil nutrient mineralization more effectively.

Physiologically, all the three types of AnM techniques induced osmotic adjustment, which improved photosynthetic activities by maintaining a higher leaf turgor potential, especially after the hypocotyl exposing stimulation is released. The improved photosynthetic activities also reflected in less hysteresis of photosynthesis and more sensitive stomata oscillation. In the leaves of the hypocotyl-exposed peanut seedlings, stomata closed more completely when water shortage was perceived and tried to open again when leaf water was in a relative balance. The osmotic potential and leaf relative water content at zero turgor (π_{IP} and ζ_{IP}) were lower in leaves of peanut plants with hypocotyl exposure treatment, suggesting that leaf turgor could be maintained to severer desiccation and contribute to stress resistance. Increased cell osmotic concentration might ensure the inward flow of water from apoplast to symplast and consequently the symplastic water fraction (ζ_{sym}) was larger in leaves of hypocotyl-exposed peanut plants, which might ensure, at least in part, the higher biochemical and physiological activities.

The stimulation of exposing hypocotyl to light at the “n” stage induced the production of superoxide radicals ($O_2\cdot^-$) and the $O_2\cdot^-$ producing rate in hypocotyls and leaves of hypocotyl-exposed peanut seedlings was significantly higher than in control. The concentration of MDA in both hypocotyl and leaf was not higher, sometimes lower in hypocotyl-exposed seedlings than in control seedlings. No increase in MDA suggested that there was no real or severe oxidative damages occurred in hypocotyl exposed peanut seedlings although the superoxide radicals were really increased. This suggested that the hypocotyl exposure treatment was just a mild stimulation instead a severe stress. The SOD activity in hypocotyls and leaves of hypocotyl-exposed peanut seedlings increased in response to the stimulation of hypocotyl exposure but POD and CAT activity were not enhanced at the early period of the exposure treatment. It was suggested that exposing hypocotyl was not a stress severe enough to induce immediate activation of POD and CAT.

Soon after the hypocotyl exposure started, anthocyanin accumulation was observed visually in hypocotyls of the young seedlings. The anthocyanin accumulation is accompanied by accumulations of soluble sugars, soluble proteins as well as the production of superoxide radicals and activation of antioxidant enzymes. The microscopic observation showed that amyloplasts were fewer in the exposed hypocotyls than in the hypocotyls grown underground. Destructive consumption of carbohydrates might occur and turn into sugar or other form of energy in the exposed hypocotyls since osmotic adjustment as well as anthocyanin biosynthesis was an energy consumable process, which provided better condition for growth of shoot and root, where biomass production was improved. It is suggested that all the consequences of the xerophytophysiological responses collaborated together to make the crop healthier through their individual function in plant growth and development.

Gdi-15 (Groundnut desiccation induced) gene is a stress-responsive gene in peanut plant. It showed homology to flavonoid 3-O-glucosyltransferase which involves in the last step of anthocyanin biosynthesis. Transcript level of *Gdi-15* gene in hypocotyl, where the exposing stimulation was directly imposed, was enhanced showing an up-regulation expression induced by the hypocotyl exposure, which was consistent with increased accumulation of anthocyamins and other osmotically active substances such as sugars, proline and soluble proteins. However, the transcript levels of *Gdi-15* gene in leaves and root of hypocotyl-exposed peanut seedlings were not activated or showed expression of a little down-regulation.

In overall, hypocotyl exposure as a stimulation did induce the up-expression of the drought responsive gene, *Gdi-15*, and the consequent osmotic adjustment and anthocyanin accumulation but caused no damage to the whole plant. This is the key point of applications of xerophytophyiology and signal transduction in plant production, with false, mild or temporary drought stimulation to induce positive regulations. In conclusion, as one of practices on the theory of xerophytophysiology, the AnM techniques, especially the modified AnM as well as transplanting seedling with extra-elongated hypocotyls as the alternative of AnM practice, were effective in inducing drought responsive genes and expected positive regulations in crop production.