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# The Effect of floodingand nutrition levels on reproductive growth stages of aerenchyma formation and ethylene production in soybean (Glycine max L)

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## Abestract

To survey the effects of flooding during the reproductive growth stages of aerenchyma formation and ethylene production in soybean cultivar DPX experiment the completely randomized factorial was in 2012 in Gorgan University of Agricultural Sciences and Natural Resources. Factors examined include nutrition levels in three levels (1 - inoculated with bacteria JaponicumBradyRhizobium 2 - non-inoculated plus nitrogen fertilizer (urea) 3 - non-inoculated without nitrogen fertilizer) and the second factor is the duration ofwaterlogging stress (0, 5, 10 and 15 days). Based on the results obtained with increasing duration of flooding stress on the plant, ethylene production increases. The slope was slowly at first and then increases exponentially. Among the nutritional treatment of ethylene production , non-inoculated plus nitrogen treatments was higher than the other two treatments, and the non-inoculated treatments without fertilizer lowest ethylene production was observed. Images of cross sections of soybean plants showed that the stress increases with the duration of flooding stress signsaerenchyma tissue formation was observed in soybean plants. 15 days of flooding treatment aerenchyma tissue formation was observed at all levels of nutrition.

**Key words**: Flooding stress, Soybean, Aerenchyma tissue, Ethylene, Inoculation with bacteria Rhizobium.

## Introduction

Soybean (Glycine max (L.) Merr)is annual plants of legumes (Leguminosae), including flood-sensitive plants in all stages of growth. Destructiveeffects of flooding stress is on the quality and quantity of agricultural production (Youn et al, 2008). Temporary and permanent flooding often occurs during plant crop cultivation. The flood situation defined bypart of the stem plant below the water surface, But if the pores are large, water-saturated soils waterlogging occurs(Kafi et al, 2008).In Flooded soils space for air was filled by water and aboutdiffusion,gas in water Ten thousand times reduced In this case, the oxygen in the rhizosphere (root environment) by roots and soil depletion and problem is hypoxia or lack of oxygen in soil(Galeshi et al, 2008).Adaptation mechanisms for the supply of oxygen to the tissues of the root flooded, including the development of air transport channels (aerenchyma) are roots of oxygen transfer from anaerobic to aerobic shoot makes possible (EmdadulHaque et al, 2010) The most obvious

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anatomical origins of crop response to soil flooding conditions Anoxia, developing extensive aerenchyma system in skin they are in the gas transmission system under stress. Aerenchyma tissue of origin may vary among species and the aerenchyma formation are involved in different physiological processes leading to the formation of three is Aerenchyma: 1 - Lysuzhnusaerenchyma in the wake of the dissolution, degradation and aging of certain cells are developed and There are more mature and developing roots and rhizomes. 2.- The Shisuzhnusaerenchyma gas space separating the cells that were previously linked together to form more stems and petioles of plants in wet areas can be seen. 3 - Akspansiuzhnusaerenchyma and the intercellular space expansion during cell division and cell enlargement without separation or death occurs (Bailey-Serres et al, 2005). species may be observed in all three types of Aerenchyma (Galeshi et al, 2008). Aerenchyma formation creates an internal channel gas exchange of shootaerobic and hypoxic roots (Shimamura et al, 2003). Air through the stomata on the leaf or lenticel on stem is inserted through the network Aerenchyma channels to reach the roots immersed in water (Thomas et al, 2005). Oxygen consumption at the root of a negative pressure gradient causes the air mass flow move to the roots. According to flooding problems occurring in the course of plant life in these conditions can be influenced by the existence of aerenchyma in roots, Because 1- In many species, aerenchymaif not all provide oxygen require at least a large part of the needed oxygen to the roots and surrounding soil the roots provides. 2- Are the roots that have aerenchyma in flood conditions are grown continuously. 3- The roots of the maize aerenchymawhen transferred to anaerobic conditions have ATP and ATP to ADP ratio greater than roots of non aerenchyma (Jackson, 2002). Aerenchyma development of hypoxic cells begins with the release of ethylene. Thus the increase in ethylene synthesis in roots under hypoxic conditions is concurrent with the development of Aerenchyma. In presence ethylene synthesis inhibitors Aerenchyma formation in flooded conditions the will be not formed. The final step in the synthesis of ethylene catalyzed 1- Amino Siliconpropane -1 - carboxylic acid (ACC) through the ethylene-forming enzyme whichThe complete absence of oxygen stops (Galeshi et al, 2008).Aerenchyma formation is essential for the growth of the root tip (He C et al, 1996). Root tip may be the place where the transfer of ACC synthase which ACC transfer to mature tissues and in aerobic conditions, ethylene synthesis and the formation of aerenchyma provides (Mano et al, 2007).Aerenchyma formation in rice roots induced genetic control because regardless of environmental conditions often found in rice roots form aerenchyma (Komatsu et al, 2010).

## Materials and Methods

The research was done in the Department of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, 2012 with factorial arrangement a completely randomized design with 3 replications was conducted as a pot outdoors. Factors considered include the nutritional levels at three levels: (1 - inoculated with bacteria *JaponicumBradyRhizobium*. 2 - Non-inoculation plus nitrogen fertilizer (urea). 3 - Non-inoculation without nitrogen fertilizer) and the second factor is the duration of flooding stress (0, 5, 10 and 15 days).flooding of soybean plants in the reproductive growth stage (R2) was applied.For this purpose, DPX soybean varieties that farmers are used as the most common variety, were used. Required for seed cultivation and development center in Gorgan were prepared from oilseeds which have the power of germination was above 90 percent. Use plastic pots, 30 cm in diameter and 27 cm in height and had proper drainage. Soil in the pots was Sandy clay loam (50% sand, 30% clay and 20% silt). At the bottom of the pot holes to drain excess water (drainage) was created. After plant establishment by eliminating the number of additional plant per pot was reduced to 3. Phenological stages of the stress with the markings 10 plants in each treatment and measure the characteristics of this plant as was. In the level first (seed inoculated with bacteria *JaponicumBradyRhizobium*) addition of inoculated seeds before planting, 50 kg ha urea was added as starter pots, soil, and in the second level (nutrient with nitrogen fertilizer) nitrogen rate of 200 kg the fourth stage was added to the soil at the third level of nitrogen nutrition treatments (no fertilizer and bacteria) from any type of fertilizer nitrogen-fixing bacteria were used. In order to flooding treatments, all treatments of the pot into the large pan that had already been built, so that the plant stems to a height of 2 cm of water swept. After each of the treatments during the flood period (5, 10 and 15 days) The pots were out from the water. To measure the amount ethylene of plastic pots in reproductive growth stage (R2) were for 24 hour in a closed space (by transparent plastic so that the soybean plants have been closed in the space) To prevent air exchange between inside and outside, in the this 24 hours of stress ethylene produced by plants as the gas enters a closed environment. After 24 hours, Indoor on the bottom (the bottom part because ethylene is lower due to the weight of the air) Ethylene samples were taken by syringe tube, by parafilm insulation was and were transferred to the laboratory. In the laboratory by of a Hamilton syringe was extracted 1 mL syringe and injected into a gas Chromatography device. Temperature, injection, detector and heat chamber (oven) 150, 150 and 100 ° C were set. Helium flow rate 9/9 ml min was used as mobile phase. After injection, the area under the curve and standard curve obtained by plotting the amount of ethylene nano-liters per gram fresh weight per hour (nL.gFW-1.h-1) was calculated.

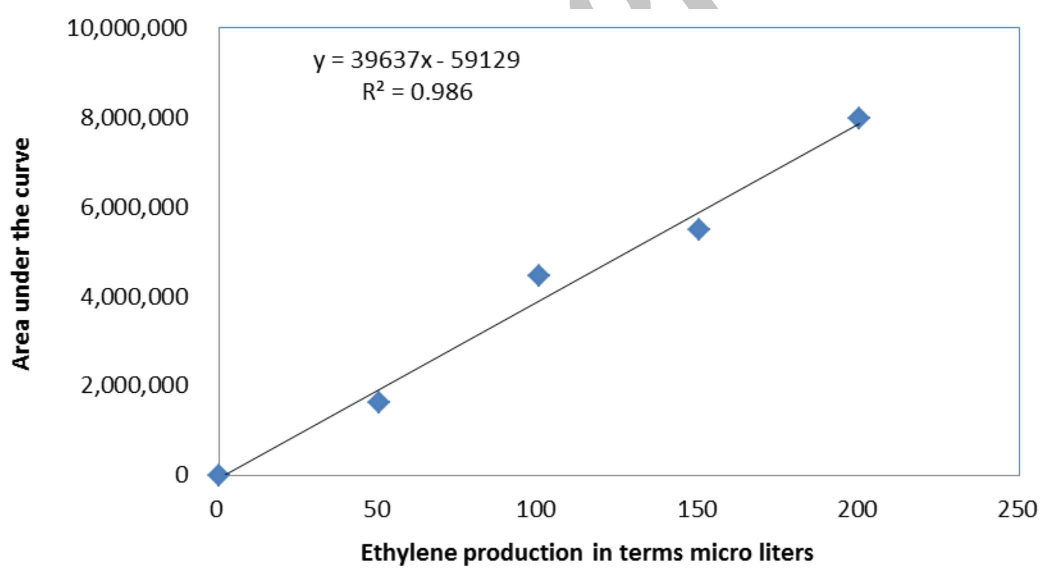



**Figure 1: Standard curves of ethylene**

Using the area under the curve obtained from samples the amount of ethylene per microliter was achieved and then turned to nano-liter. Investigate was doneaerenchyma formation in the stem after the stress of soybean plants with roots were out from the pots and washing were transported to the laboratory. In the laboratory by the scalpel of the upper cross section of soybean roots (curb orputeal) form aerenchyma was prepared for investigate, Then by the frisette were fixed

Cross section were And were photographed using a microscope cameraand then to investigate the cross-sectional area of aerenchyma formation was discussed.

## Results and Discussion

### Ethylene

The effect of different periods of flooding at different levels of nutritional stress on ethylene levels in the plant tissues of soybean in Figure (2) is shown. The highest rate of ethylene production in non-inoculated plus fertilizer treatments were flooding in 15 days and ethylene lowest non-inoculated treatments without fertilizer (Figure 2). According to the table (1) gradient of ethylene production nutritional levels 1 % probability level was significant, which production Ethylene treatment plus fertilizer non-inoculated was the highest slope and the minimum slope for ethylene production without fertilizer treatments were not inoculated (Figure 2). Ethylene production rate increased with increasing duration of flooding stress on nutritional levels rose. Figure (2) is showed, the beginning of flooding stress (5 and 10 days) the amount of ethylene produced by the plant are very low, but at 15 days of flooding stress ethylene production at the plant will be faced with a jump that which this could be a mechanism for plant adaptation to flooding stress is encountered and by the production of aerenchyma tissue to deal with stress it can irritate. Kashani and MalekMohammadi (2008) in pepper plant ethylene levels increased with increasing duration of flooding stress were reported. Ahmed et al (2002) in mung bean plants increased levels of ACC (ethylene precursor in the plant) were reported with increasing flooding stress.

**Table 1 the estimated parameters (a, b) and confidence coefficient (R2) of the fitted equations for ethylene**

| nutritional levels | a±SE | b ±SE | $R^2$ |
|---|---|---|---|
| Non-inoculation plus nitrogen fertilizer | .82±.25 | .22±.2 | .99 |
| inoculated with bacteria | .53±.23 | .22±.02 | .99 |
| Non-inoculation without nitrogen fertilizer | .34±.26 | .24±.05 | .99 |

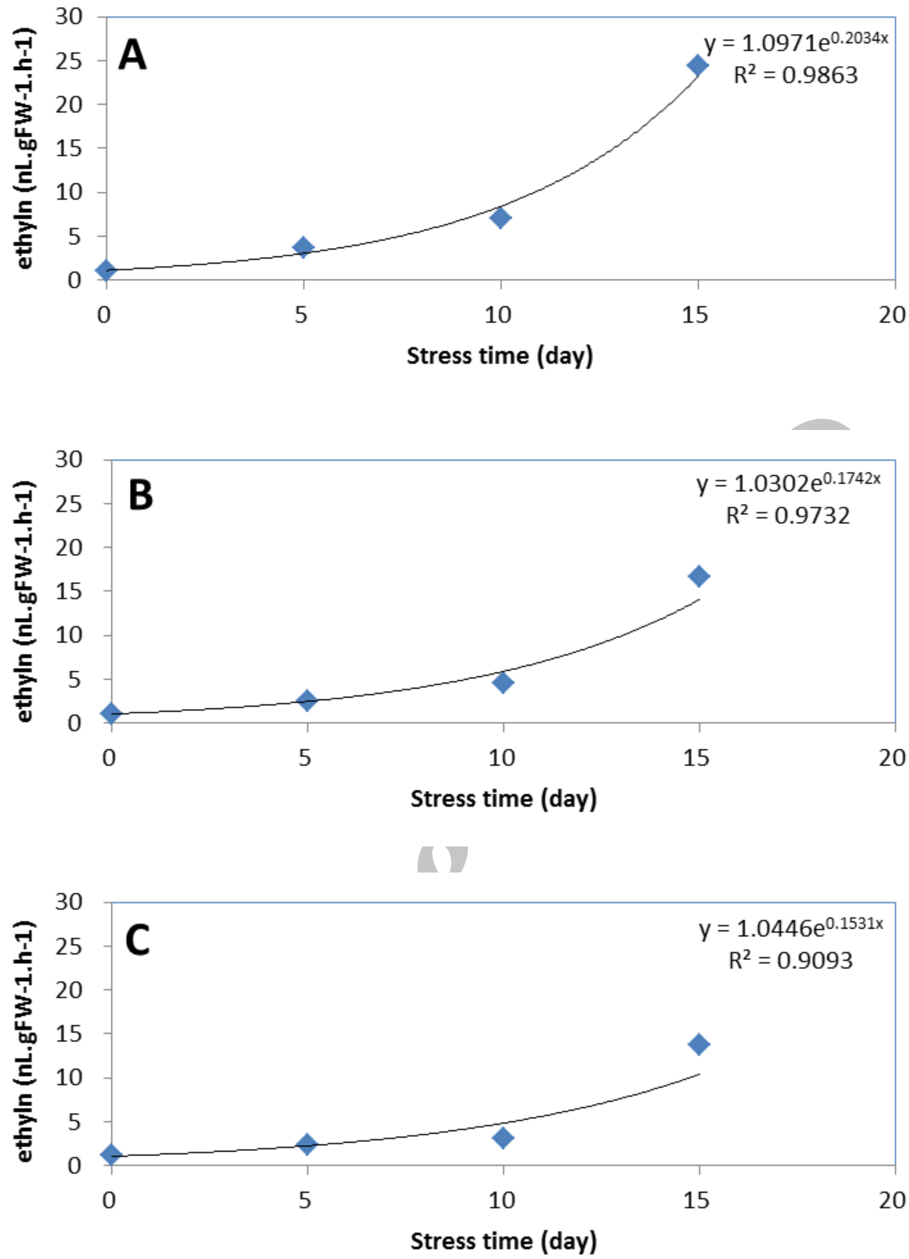
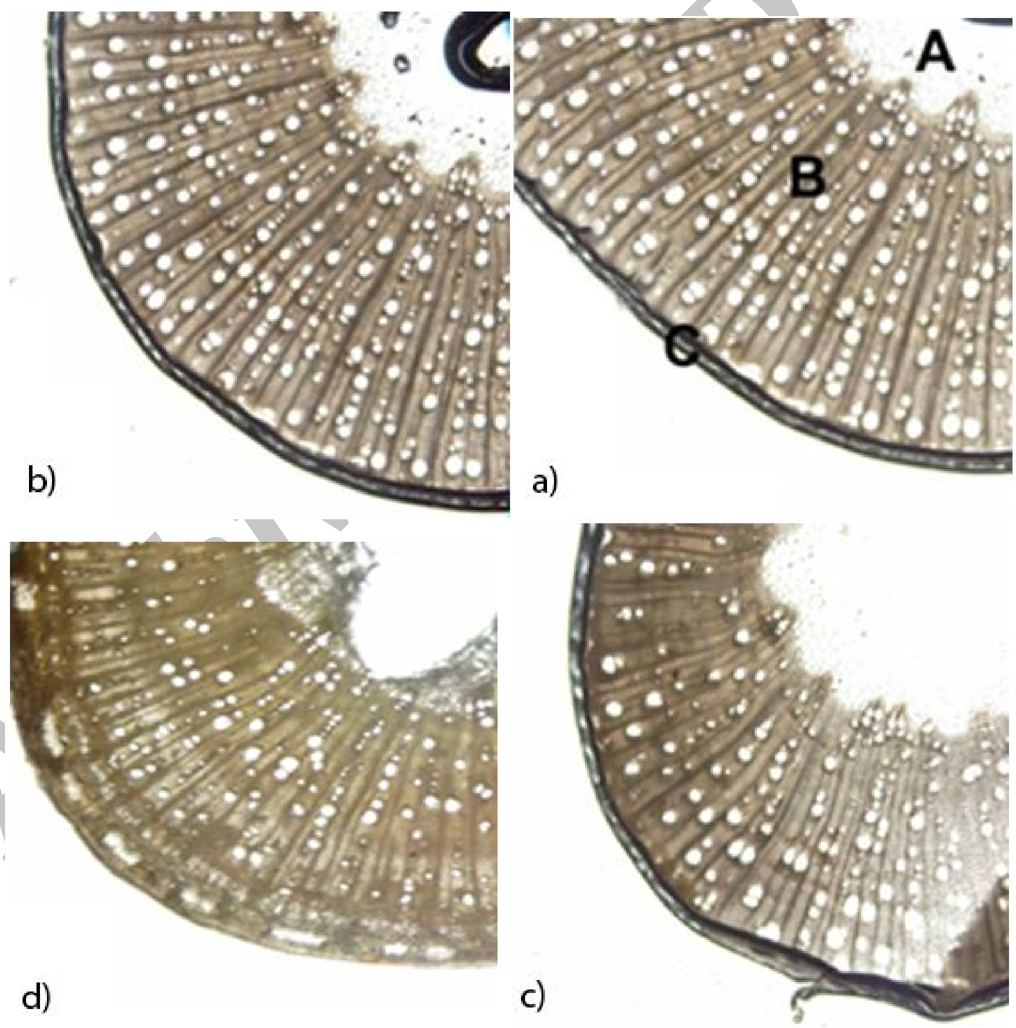
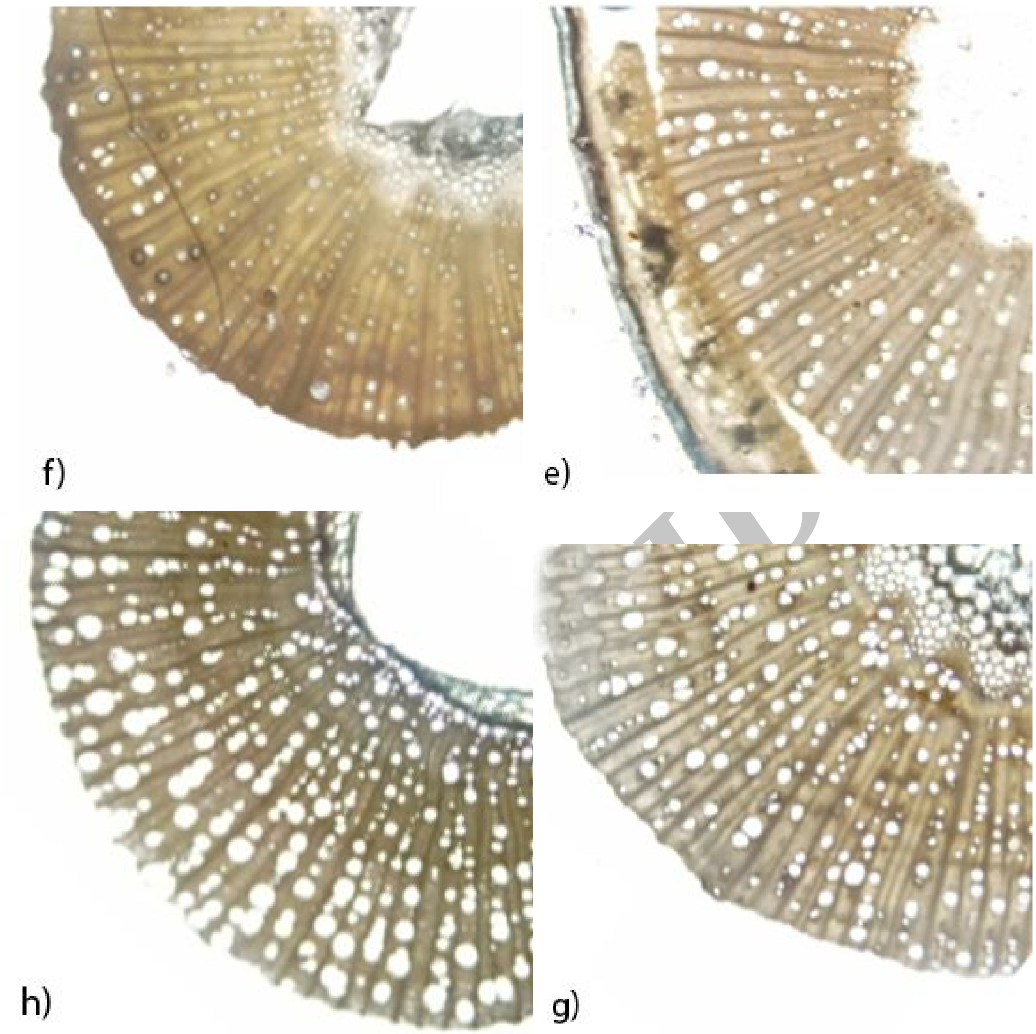



**Figure 2 Effects flooding stress (0, 5, 10, 15 days) on the rate of nutrition of ethylene at different levels (A: non-inoculated fertilizer plus B: inoculation without fertilizer C: Non-inoculation without fertilizer)**

### Aerenchyma tissue

One of the characteristicsinvestigated of this survey formed aerenchyma tissue in was response to flooding stress. The cross section of soybean stem, the stress was produced, It was clear that the early symptoms of aerenchyma tissue in 15 days, there was of flooding at all levels of nutritionAerenchyma formation in 5 and 10 days, there were no signs of flooding were consistent with an increase in ethylene production.One plant adaptations to cope with flooding stress aerenchyma formation in shoot tissue (leaf and stem) and underground parts (roots) of the plant, When flooding is so rooted in its oxygen from the atmosphere to provide. Lisuzhnusaerenchyma tissue (aerenchyma first emerged in the tissues of parenchyma tissue), there is the destruction of parenchyma cells. Thomas et al (2005) also formed in soybean root aerenchyma tissue two weeks after it was flooded stress on the plants, they report in three weeks to complete the development of flooding stress observed aerenchyma tissue. Shimamura et al (2003) Complete development of aerenchyma tissue three weeks afterfloodging stress in soybean were observed. Yan et al (2008) in soybean aerenchyma tissue, the first signs were observed when the plant was flooded under two weeks.




f)
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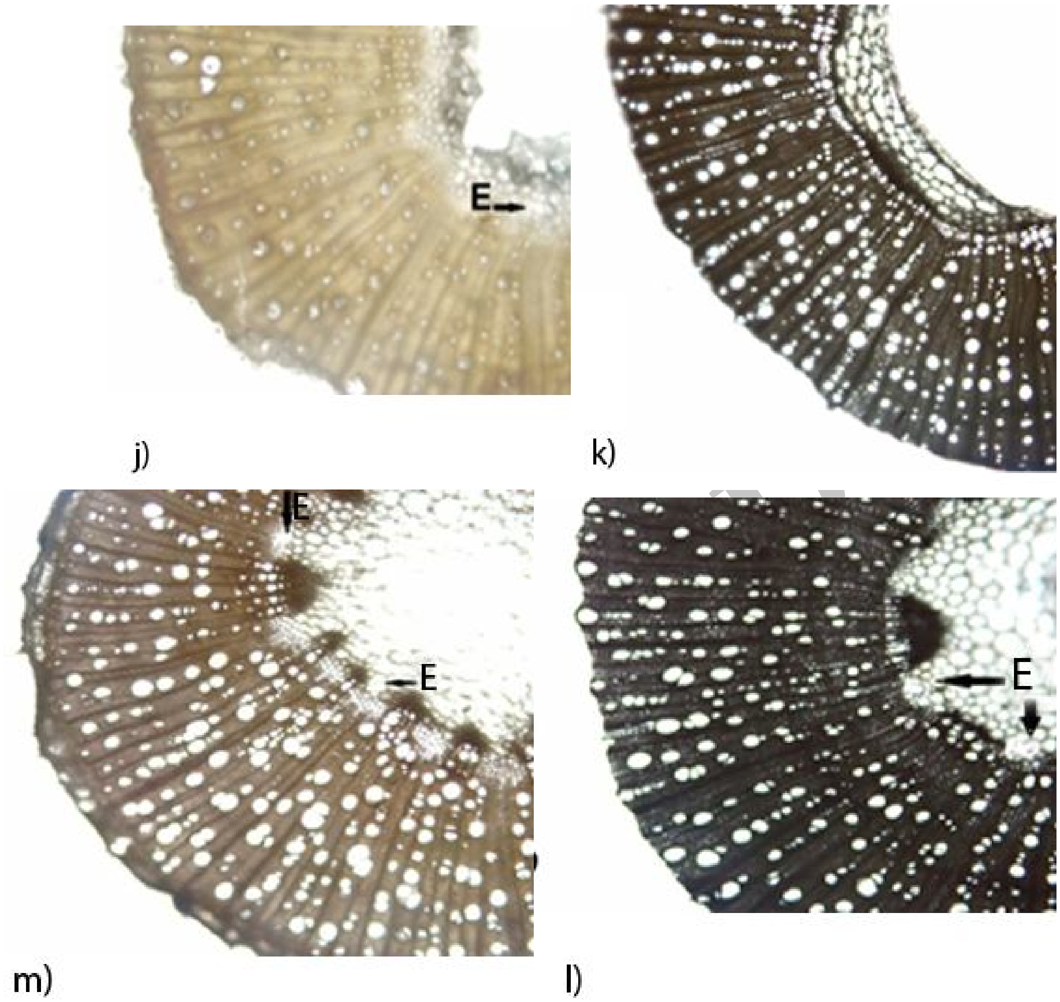


**Figure 2 Cross section of flooding at different levels of nutritional stress during soybean plant (a, d, g, j Cross-section in order to Non-inoculation plus nitrogen fertilizer levels at zero, 5, 10 and 15 days of flooding, b, f, h, m Cross-section in order to inoculated with bacteria levels at zero, 5, 10 and 15 days of flooding, Cross-section in order to c, e, k, I Cross-section in order to Non-inoculation without nitrogen fertilizer levels at zero, 5, 10 and 15 days of flooding). A: pith, B: C: cortex. In Forms j, I, m The letter E shows early signs of aerenchyma tissue formation.**

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