RJOAS: Russian Journal of Agricultural and Socio-Economic Sciences
ISSN 2226-1184 (Online) | Issue 5(137), May 2023

UDC 633; DOI 10.18551/rjoas.2023-05.11

NITROGEN METABOLISM OF SOYBEAN AT SATURATED SOIL CULTURE AND WATERING CULTIVATION OF FARMER'S SYSTEM IN CONDITIONS OF TIDAL LAND

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ABSTRACT
This research aimed to study the effect of the water-saturated cultivation system of Tidal land on nitrogen metabolism in soybean. Treatment was growing plants on benches, at the different water levels of trenches-which were 15 cm, 20 cm, and 25 cm from the soil surface-arranged in a randomized block design with three replications. The results showed that the height of standing water in the trench affected the soil water, leaf proline, and N content. The water level of 15 cm had a higher soil water potential (-0.48 MPa), the proline content of 1.52 mol/g fresh leaf weight, and the N content of leaves was 2.6% than those of 20 cm and 25 cm. The lowest N content and leaf chlorophyll content were obtained at a height of water level of 25 cm, namely 1.79% leaf fresh weight and 1.74% leaf fresh weight, respectively, but, higher proline content was found at 2.47µmol/g fresh leaf weight. Thus, both the water level of the bench of 15 cm and 20 cm had better nitrogen metabolism. Plants grown at the benches of 25 cm can still withstand low soil water content, proven by their highest leaf proline content. The increase in proline value in soybeans under stress conditions was caused by de novo synthesis, not due to protein degradation. The mechanism of nitrogen metabolism in soybean (C3) under mild stress conditions is postulated to be the same as nitrogen metabolism in corn (C4).

KEY WORDS
Nitrogen metabolism, soybean, saturated soil culture, tidal land.

One of the efforts to increase soybean production is through an area expansion program to increase the planted area. However, the implementation of this program encountered obstacles because fertile land was increasingly limited, either due to competition for land use for other commodities or due to land conversion. Therefore, the expansion of the planting area is directed at sub-optimal land use such as tidal land. Based on the inundation pattern or the range of the tide, overflow type C is not flooded but the groundwater depth at high tide is less than 50 cm (Ar-Riza, 2008). Soybean cultivation which is usually done by farmers is to provide intermittent irrigation (conventional) which is carried out every 2 or 3 weeks, in this study, it is called Watering Cultivation (WCV). Intermittent provision of water interferes with plant growth because while in a state of dryness it will experience stress and when water is given a recovery occurs, but with watering intervals of 2 - 3 weeks, before recovering the plant will experience drought stress again. This condition affects plant metabolism, including nitrogen metabolism.

Tidal land is a sub-optimal land because it has limitations that require careful handling such as the presence of a layer of pyrite (FeS_2) which when oxidized will produce sulfate compounds that are harmful to plants. The right touch of technology can potentially increase the productivity of tidal land. Irrigating (flooded) the trench, which is called Saturated Soil Culture (SSC) technology, provides water that irrigates plants continuously through waterlogging in ditches. Certain water levels are expected to maintain the depth of the groundwater surface and the rhizosphere or root area is always in a reductive atmosphere so that pyrite (FeS_2) is not oxidized. Under stress conditions, plants commonly react by the accumulation of compatible solutes, such as proline, in cells, which improves environmental stress tolerance (Ashraf and Foolad, 2007; Hong et al., 2000; Ramanjulu and Sudhakar, 2001).

Water standing in the trench, causes the plants to absorb less soil nitrogen than regular irrigation. Under low soil nitrogen conditions, N fixation is a major source for plants (Ramanjulu and Sudhakar, 2001; Troedson et al., 1985). SI5 cultivars with inundation in the trench without nitrogen fertilizers got 74% nitrogen from the air, whereas with ordinary irrigation only 54%. Although the formation of large nodules is a characteristic of soybean plants that get inundated in the trench, the presence of nodules is not important for increasing yields. This was shown from the yield of plants without nodules but fertilized with N was higher than the nodule plants that were not fertilized (Troedson et al., 1985).

There are few studies providing information on whether the nitrogen metabolism of soybean grown in irrigation in a trench can proceed and that of continuous water in a trench. The optimum water level of inundation was also important information to be seeking. This research was conducted to study the effect of wet cultivation; inundation in trenches on soybean nitrogen metabolism grown in type C tidal land.

METHODS OF RESEARCH

This research was conducted in Simpang Village, Berbak District, East TanjungJabung Regency. A randomized block design was used as an environmental design. Saturated soil culture in this study maintained the height of the water in the trench, namely, 15 cm, 20 cm, and 25 cm below the bench's soil surface. As control was the farmer's usual cultivation system (farmer's version) hereinafter referred to as watering cultivation (WCV), which is intermittent watering which is usually done every 2 or 3 weeks. In this study, 3 farmer groups (FG) were determined as WCV actors (FG1, FG2, and FG3). Each treatment was repeated 4 times so the number of experimental plots was 12 units for SSC and 12 units for WCV. Soybeans were planted with 30 x 20 cm spacing in 3 x 2 m beds.

Environmental observations in the form of soil moisture content were carried out 6 weeks after planting, in the center of the plot at a depth of 5-10 cm using the gravimetric method, and converted into groundwater potential. The measurements were carried out at noon (13.00), and in the afternoon (17:00). Observations on the main variables were carried out at the age of 6 weeks after planting (WAP) including:

(1) Nitrogen content of fresh leaves, measured by the Kjeldahl method;

(2) Leaf chlorophyll content, carried out using a spectrophotometer at a value of absorbance of chlorophyll solution at wavelengths of 663 nm and 645 nm. Then calculated using the formula: leaf chlorophyll = 17.3 A645 + 7.18 A663 mg/l (Guofa, 1990);

(3) Proline content, measured following the Bates method (Harbone, 1960) using a spectrophotometer at a wavelength of 520 nm;

(4) Amino acid testing using the Ultra Performance Liquid Chromatography (UPLC) method. Amino acid analysis using UPLC consists of several stages, namely. Samples were weighed as much as 0.1 g crushed and put into a closed test tube. The sample solution was added with 5-10 mL of 6 N HCl, hydrolyzed in an oven at 110°C for 22 hours, then cooled at room temperature and transferred to a 500 mL volumetric flask. Then added aqua bides up to the mark and filtered with a 0.45 L filter and a 10 L pipette, added 70 L of ACCQ- Fluorine Borate, and vortexed. Then 20 L of Flour A and reagent was added and vortexed and allowed to stand for 1 minute and incubated for 10 minutes at 55°C. then injected into UPLC as much as 1 L under chromatographic conditions using ACCQ-Tag Ultra C18 column, temperature 49°C, mobile phase composition system gradient detector PDA, flow rate 0.7 L/min, and wavelength 260 nm (Gianto et al., 2017).

Soil water potential data were analyzed by the analysis of variance (ANOVA) and then continued with the least significant difference test (LSD) to see the differences between treatments. Laboratory analysis of the Nitrogen content of fresh leaves, Leaf chlorophyll content, proline content, and amino acid was carried out. The data on the nitrogen of fresh leaves, leaf chlorophyll content, proline content and amino were displayed in graphical form and they are presented in narrative form.

RESULTS AND DISCUSSION

The water potential in plots treated with a height of inundation of 15 cm from the plot surface averaged -0.48 MPa. The soil water potential (ψs) in plots with a height of inundation of 20 cm averaged -0.63 MPa, and in a plot with a height of inundation of 25 cm averaged -1.55 MPa (Figure 1A). This indicated that the soil water content in the plots with a height of inundation of 15 and 20 cm is higher than that of 25 cm, meaning that plants in plots with a height of water 25 cm from the surface of the plot can experience a water deficit. The results of the calculation of the ψs showed that the average ψs at SSC was -0.98 MPa while at WCV it was higher at -1.78 MPa (Figure 1B). Thus, WCV has more potential to cause stress effects on plants.

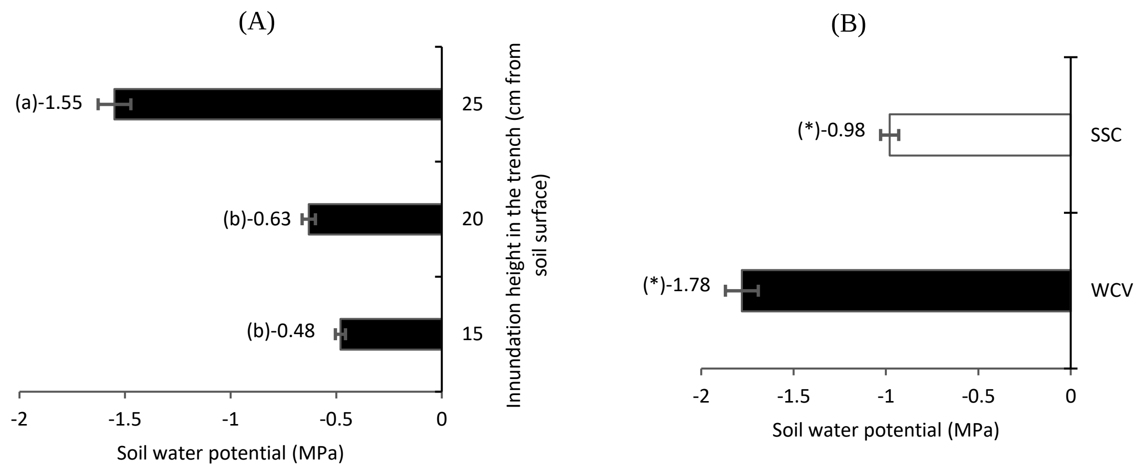

Figure 1 – Soil water potential of benches with various water levels in trenches (A), the soil water potential of saturated soil culture (SSC) and watering cultivation (WCV) (B) in tidal land.
Note: The numbers followed by the same letter are not significantly different according to the LSD test at the 5% level. The (*) sign indicates a significant difference based on the T-test at a 5% significance level.

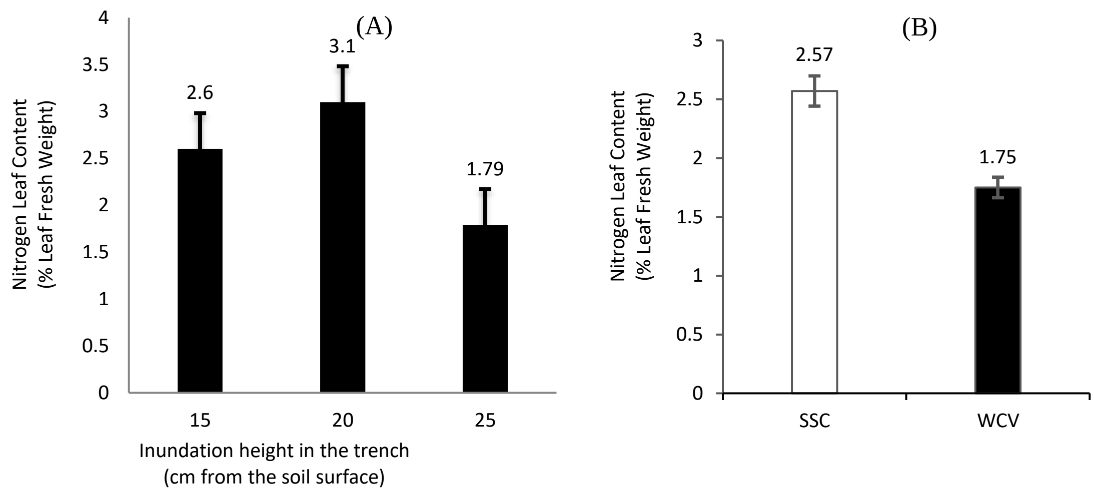

Figure 2 – Leaf Nitrogen content based on the height of the puddle in the trench (A) and nitrogen content in SSC and WCV (B) of soybean cultivar Anjasmoro

In this experiment, we found that the difference in height of standing water in the trench in the saturated water cultivation system affected the nitrogen content of the leaves. The highest nitrogen content was achieved at a water trench of 20 cm below the soil surface (3.1% leaf fresh weight) followed by a water trench of 15 cm (2.6% leaf fresh weight). Both showed higher nitrogen content than that of 25 cm from the soil surface (Figure 2 A). The nitrogen content of the leaves differed significantly according to the type of cultivation. In SSC, leaf nitrogen content was higher at 2.51%, while N content in WCV was lower (1.75%) (Figure 2 B).

The lowest chlorophyll content was obtained at the treatment at 25 cm (1,74 mg.g^{-1} leaf fresh weight) and the highest was at 20 cm (2,7 mg.g^{-1} leaf fresh weight), followed by 15 cm (2,24 mg.g^{-1} leaf fresh weight) of water from the trench surface (Figure 3 A). The chlorophyll content in SSC was higher than the chlorophyll content in WCV (Figure 3 B).

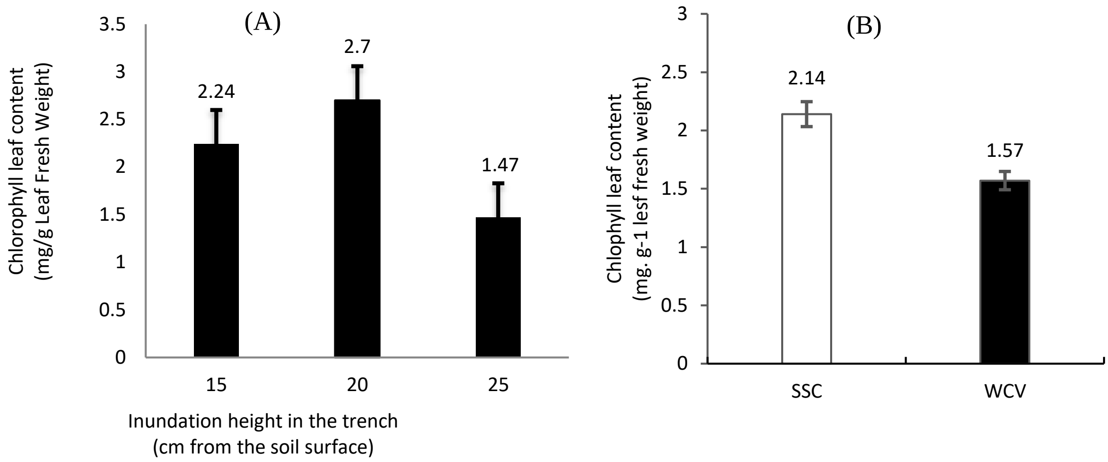

Figure 3 – Leaf chlorophyll content based on the height of waterlogging in the trench (A); Leaf chlorophyll content in SSC and WCV (B) of soybean cultivar Anjasmoro

The water levels in the trenches in the saturated soil culture affected the proline content of soybean plant varieties Anjasmoro. The highest proline content was at the plant leaf at the bench with a water level in a trench of 25 cm (2,47 μmol.g^{-1} leaf fresh weight). The proline content of plants on the water level of 15 cm and 20 cm from the soil surface was 1,52 μmol.g^{-1} leaf fresh weight and 1,57 μmol.g^{-1} leaf fresh weight (Figure 4 A).The proline content in SSC was lower at 1.8 μmol.g^{-1} leaf fresh weight, while in WCV it was higher at 2.8 μmol.g^{-1} leaf fresh weight (Figure 4 B).

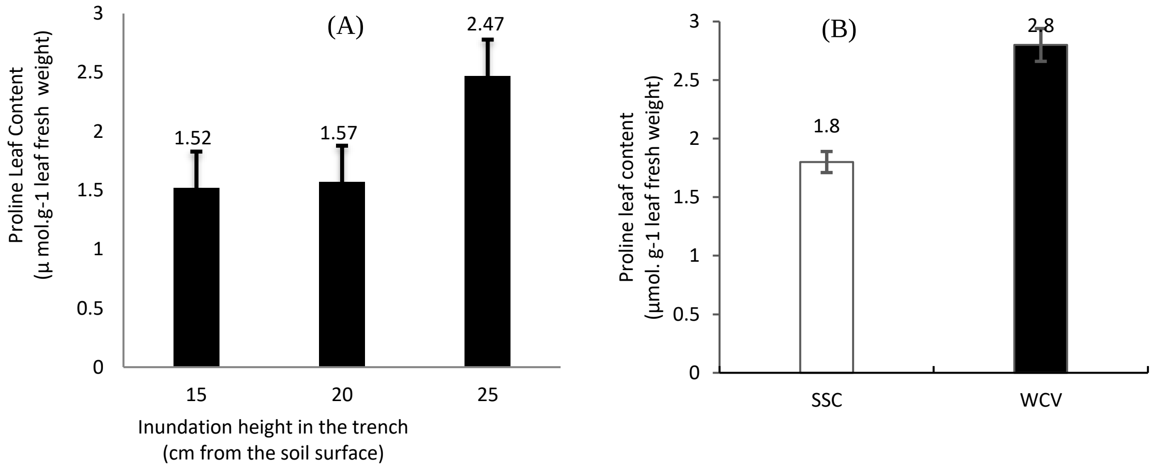

Figure 4 – Leaf proline content based on waterlogging height in the trench (A) and based on SSC and WCV (B) of soybean cultivar Anjasmoro in tidal land

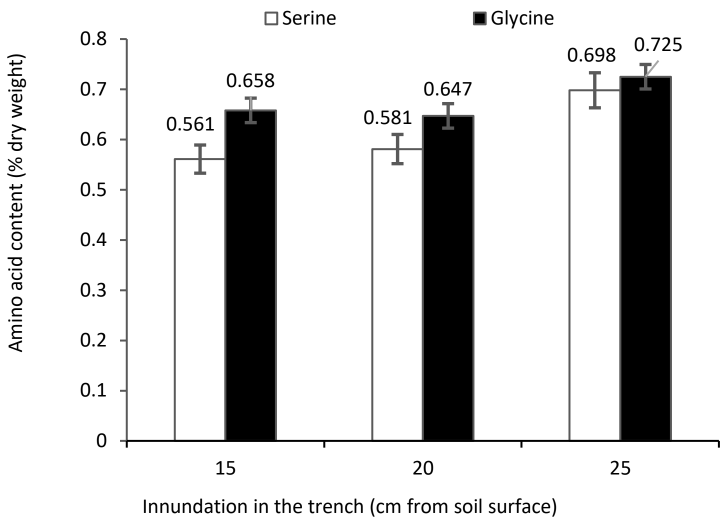

Figure 5 – The amino acid content of soybean varieties Anjasmoro at three heights of puddles in ditches

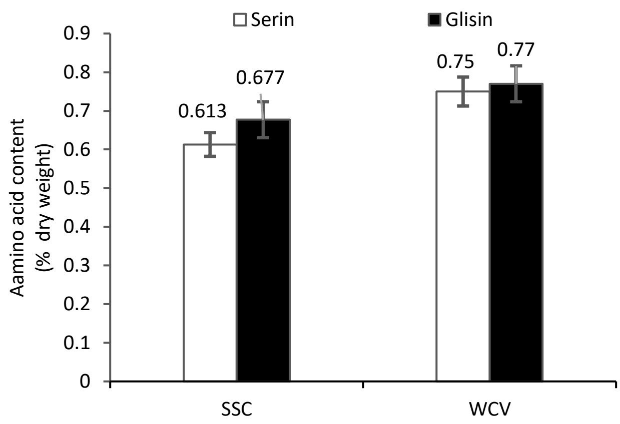

Figure 6 – The amino acid content of soybean varieties Anjasmoro based on SSC and WCV

The height of standing water in the ditch affects the amino acid content of soybeans of the Anjasmoro variety. At the height of the puddle in the trench 15 cm and 20 cm from the soil surface, the amino acid content was relatively the same, both serine and glycine. However, at the height of the puddle in the ditch 25 cm from the soil surface, the amino acid content showed an increase (Figure 5). Furthermore, in a saturated water culture (SSC) the value of amino acid content was lower (serine 0.613% dry weight (DW) and glycine 0.677% DW), while in watering cultivation (WCV) the values of these two amino acids showed an increase, each 0, 75% DW for serine and 0.77% DW for glycine (Figure 6).

DISCUSSION OF RESULTS

Type C tidal areas are generally unaffected by tides. It's just that the groundwater level is too high, so it is not suitable for soybean growth. Reduction of the groundwater level was achieved by forming a trench around the planting area. The depth of the trench was made different so that the water level is 15, 20, and 25 cm from the ground surface. The water potential in a plot with an inundation of 25 cm is ψs = -1.55 MPa and WCV (ψs = -1,78 MPa) (low soil water content). These conditions are thought to cause mild water stress effects but affect metabolic processes in plants, including nitrogen metabolism. Soybean growth and yield with inundation in trenches were increased due to nitrogen fixation and root growth above the ground water table. Many reported that inundation in trenches or wet cultivation can increase soybean yields in the field by up to 20% (Ramanjulu and Sudhakar, 2001; Bates et al., 1973; Manwan et al., 1990) and to 80% (Cooper et al., 1993).

Generally, in conditions of low soil water content, the availability of nutrients in the soil solution is also low, including nitrogen elements. In addition, with low soil water content plants will experience water shortages. Generally, plants that lack water have their leaf stomata closed so that the rate of transpiration decreases. The decrease in the rate of transpiration causes the movement of water into the plant (mass flow) to be smaller. This can lower nitrogen content in plants because these nutrients are absorbed through a mass flow mechanism. Water deficit can reduce the absorption, accumulation, partition, and use of nutrients in plants. To mitigate the effects of this stress, plants produce osmolytes, such as proline, which may assist in preventing nutrient deficiencies under water deficiency (Indradewa et al., 1993).

Adisarwanto (Cacefo et al., 2021) found that lower leaf N content with inundation in trenches persisted between 28 and 42 days after initiation. Nodule biomass continued to increase rapidly during acclimatization by inundation in the trench. Saturated conditions (pooling in ditches) at the age of 15-30 days are ideal conditions for increasing the number of nodules. Nitrogen metabolism is known to have a close relationship with photosynthesis. In this case, the light reaction from photosynthesis provides energy to reduce nitrate to ammonium, one of which is reduced by the enzyme nitrate reductase. Furthermore, the formation of chlorophyll involves the citric acid and amino acid cycles. The combination of the two will produce levulinic amino acids as an intermediate in the formation of chlorophyll. Since the nitrogen content in plants at a water height from the trench of 25 cm was low, the amino acid which is the precursor for the formation of chlorophyll decreases, resulting in a reduction in the formation of chlorophyll. Soil water content (soil water potential -1.55 MPa) was found on a plot with an inundation of 25 cm (See Figure 1 A). This condition resulted in decreased photosynthetic activity and suppressed primary metabolism. Thus, the formation of carbohydrates as one of the chlorophyll-forming factors is reduced. Energy and reducing power for nitrate reduction come from cellular metabolism, namely carbohydrate respiration (Adisarwanto, 2005). Whereas photosynthesis increases the supply of carbohydrates and NADH needed for nitrate reduction resulting from carbohydrate respiration.

One of the important mechanisms exerted by higher plants under abiotic stress is the accumulation of compatible solutes, such as proline. The most common response carried out by plants in conditions of water shortage is the accumulation of osmolyte compatible compounds which are neutral organic compounds that have active osmotic abilities. These compounds function to protect plants under stressful conditions (Bidwell, 1979). The accumulation of compatible osmolytes can decrease the water potential in cells (Chutipaijit et al., 2009; Mathius et al., 2004). The presence of great amounts of proline in unstressed citrus trees occurs when this amino acid accumulates far more than the demands of protein synthesis (Mathius et al., 2004). Beside it, the proline accumulation rises in response to water deficit (Taylor, 1998; Kishor et al., 2005; Nolte et al., 1997; Campos et al., 2011; Girardi et al., 2017).

The amino acid proline is the most widely distributed osmolyte compatible. Proline synthesized during periods of water shortage can act as a provider of organic nitrogen which is useful in the cell recovery process. Proline degradation in mitochondria is directly related to the electron transport system and ATP production. It can improve the energy status of cells recovering from water shortage conditions (Arias-Sibillotte, 2019). Based on the results of our laboratory analysis, it can be seen that there are significant differences in proline contents. The highest was achieved in plants with a water level inundation of 25 cm and the smaller at a water level inundation of 15 cm and 20 cm from the trenches' surface.

The low proline content of plants on 15 cm and 20 cm from the soil surface could be justified since there is still sufficient water available for plants so that plants do not have to accumulate osmolyte compatible compounds which in turn can reduce water potential in cells that allow uptake additional water from the environment. The high accumulation of proline on 25 cm was thought to be because proline in plants with low water availability is synthesized as a consequence of cell osmotic regulation by increasing levels of soluble compounds in cells so that the intracellular osmotic potential is lower or at least proportional to the potential for intracellular osmosis around the cell. Several research results showed an increase in proline levels under conditions of low water availability, including spinach and tomatoes (Lawlor, 2002) and corn plants (Umebese et al., 2009).

Proline accumulation is a common response of plants to water stress (Chutipaijit et al., 2009; Lawlor 2002; Heidari and Moaveni, 2009; Darusman et al., 1991; Ganesh et al., 2009; Hamim et al., 1996). In response to water deficit, plants synthesize and accumulate some compounds that perform protective functions, in addition to helping to maintain plant metabolism, growth, and development. An example is the amino acid proline, which acts as an osmoprotectant, stabilizing cell structures, and enzymes, eliminating reactive oxygen species (ROS) and maintaining the redox balance in adverse situations Hamim et al., 2008). Proline can act as an osmolyte compatible, as a membrane and enzyme protective agent, as a temporary transit site for organic nitrogen, and act as a free radical scavenging agent (Meena et al., 2021). Proline accumulation is a plant's effort to maintain cell turgidity (Hare et al., 1999). Proline in plants is synthesized as a consequence of cell osmotic regulation, which is caused by low water availability. This condition will spur some plants to increase their respiration rate to produce ATP which is used to activate cells under stress and dissolved osmotic substances that can reduce the osmotic potential of cells, thereby increasing cell water uptake which will simultaneously increase turgidity and activity (Meena et al., 2021).

Nitrogen assimilation is closely related to carbon metabolism in biochemical pathways in plants. Nitrogen assimilation, especially nitrate reduction, is a highly energy-dependent reaction (Bloom et al., 2010). Nitrate reduction in leaves can use the reducing power derived from photosynthesis and is more efficient than the reduction that occurs in roots under water stress situations (Gonzalez-Dugo et al., 2010). Thus, nitrogen assimilation acts as an important alternative electron store and overexcited energy to minimize photoinhibition and photodamage of photosynthesis. In addition, it stimulates CO_2 assimilation under conditions of stomatal limitation caused by osmotic pressure (Yi et al., 2014). Stomata control the exchange of air and gases between the leaf and the surrounding air. The trade-off between water loss and gas exchange is critical for plants to maintain photosynthetic capacity under water deficit conditions (Manzoni et al., 2013). Although partial closure of stomata is very important to prevent water loss to maintain water balance in plants, stomata closure increases the diffusion resistance of CO_2 from the air to the intercellular air space (stomatal conductance), as well as from the intercellular air space to the carboxylation site (mesophyl conductance). To date, the relative importance of gs and gm in photosynthesis is controversial (Niinemets et al., 2009; Xiong et al., 2015; Barbour and Kaiser, 2016).

Water stress has been stated to occur at a height of standing water in a ditch 25 cm from the soil surface and WCV has been widely reported that water stress can cause the breakdown of proteolytic proteins and consequently lead to the accumulation of amino acids (Akinci and Sösel, 2012). However, the mild stress in our study that occurred at the height of waterlogging in the ditch and on WCV in soybean variety Anjasmoro was not suspected to cause a decrease in soluble protein. This is indicated by the increase in amino acids, both serine, and glycine. Amino acid variations resulting from mild stress at high levels of waterlogging in a ditch 25 cm from the soil surface and at WCV are more likely to reflect changes in N uptake and assimilation, and amino acid turnover, for example, proline synthesis (Walch-Liu et al., 2005).The results of our study are in line with the results of previous studies on maize plants which were not subjected to stress more fixation in organic acids than amino acids, but under conditions of carbon stress, there was an increase in amino acids, serine, and glycine. Thus, it was postulated that the pattern of nitrogen metabolism in soybean (C3) and maize (C4) did not show any difference under water stress conditions. Another factor that may cause variations in amino acids in plants experiencing the effects of water stress is a decrease in nitrate reductase activity. This decrease was probably due to the low nitrate translocation in the xylem (Shaner and Boyer, 1976). It remains unclear whether changes in nitrate reductase activity lead to loss of enzyme activity or increase the rate of enzyme degradation. However Bogger and Stewart (1976) proved that increased proline resulted in de novo synthesis and not protein degradation.

CONCLUSION

The height of standing water in the trench in a saturated water culture system was environmental factors affecting nitrogen metabolism, proline content, N content, and leaf chlorophyll content of soybean plants grown on tidal land. The higher the water level in the trench from the soil surface, the lower the N content and leaf chlorophyll. The lowest leaf N content and leaf chlorophyll content were obtained at a height of standing water in a trench 25 cm from the soil surface, namely 1.79% leaf fresh weight and 1.74% leaf fresh weight, respectively. However, it produced the highest proline content, which was 2.47µmol/g fresh leaf weight. The increase in proline value in soybeans under stress conditions was caused by de novo synthesis, not due to protein degradation. The mechanism of nitrogen metabolism in soybean (C3) under mild stress conditions is postulated to be the same as nitrogen metabolism in corn (C4).

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