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Purple Nutsedge (*Cyperus rotundus* L.) Interference and Drought Effect on Proline Accumulation in Soybean (*Glycine max* L.) Leaves

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1. INTRODUCTION

Seed of soybean (*Glycine max* L.) is a food of vegetable protein source that is widely recognized by society. In generally, soybeans crop planted at the end of the rainy season, so that the imperfect management potentially lead to drought and weed interference occurring simultaneously. The adverse impact of weeds on crop directly through the growth factor competition and alellopathy.[1,2] Competition led to a decrease in the level of water availability,[3] whereas alellochemicals synthesis stimulated by competition and low soil moisture.[2,4] Purple nutsedge (*Cyperus rotundus* L.) is one of the important weeds in soybean.[5,6]

A multiple stress should be seen as a new form of stress that is different from each single stress. It is caused by interaction responses on a multiple stress.[7,8] The interaction responses on multiple stress caused by the centralization response to various environmental stress in plants.[9] Multiple stress effect can be seen as a percent (%) cumulative effect, which is the ratio between the percent of multiple stress effect with the percent of single stress effect that cause most the changes in the parameters measured. If the percent value of the cumulative effect of less than 100%, the multiple stress character of cross adaptation which means that one stress causes decreased sensitivity of plants to the other stress, whereas if the percent value of the cumulative effect of more than 100%, the multiple stress character of cross synergism which meaning that one stress causes increased sensitivity to other stress.[10]

Drought is defined as a decrease of soil water potential which causes the plant is difficult to absorb the water. One of the tolerance mechanisms by plants to drought was accumulated of proline can act as osmoregulator to lower water potential in the plant cells so that the plant can still absorb water from the soil.[11–13] In the under environmental stress conditions, proline can also serve as a non-enzymatic antioxidants that can react with some of Reactive Oxygen Species (ROS) form.[14] The purpose of this study was to examine the multiple stress effect of purple nut sedge interference and drought on the proline accumulation in the soybean leaves and determine the cumulative effect character

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2. EXPERIMENTAL DETAILS

This research was designed using Completely Randomized Design (CRD) with two factors, i.e., drought [P_0 = control, fraction of Ttranspirable Soil Water (FTSW) 1, P_1 = mild drought FTSW0.5 and P_2 = severe drought, FTSW0.25] and level of purple nutsedge interference [T_0 = control, 0 purple nutsedge, T_1 = three purple nutsedge and T_2 = six purple nutsedge]. Each treatment unit was made in five replicated. Soybean seeds were selected based on uniformity of seed size, whereas purple nutsedge tubers were selected based on uniformity of tuber weight. Purple nutsedge tubers had sprouted and only one and two buds were used. Seedlings of soybean and purple nutsedge were planted at the same time in plastic pots diameter 25 cm, containing 3 kg of latosol soil with base fertilizer (1 g TSP; 0.5 g KCL and 0.3 g urea). Each pot was planted with one soybean seedling and three or six of purple nutsedge tubers according to the treatments. Drought treatment was started two weeks after planting and terminated three weeks after treatment. Drought treatment was determined based on the value of FTSW.[15, 16] Quantification of drought stress treatment was obtained by measuring the total weight of pot and plants. Watering was performed every day and the water volume used was determined by weighing the pot and its contents until the total weight was equal to the treated unit.

Determination of proline accumulation based on Bates et al. protocol with some modifications.[17] Soybean leaf samples weighing 0.15 g crushed with a mortar in liquid nitrogen and homogenized with 3 ml of acid sulfosalisilat 3%, then centrifuged at a speed of 8000 g at 4 °C for 10 minutes. Supernatant was separated as an extract of proline. Two ml of extract of proline added with 2 ml of ninhydrin acid and 2 ml of glacial acetic acid 100%, then reacted at 100 °C in a water bath for 60 minutes. The reaction was stopped by inserting test tube into icebath for 20 minutes, then added 4 ml of toluene and then shaken in a vortex for 20 seconds. Furthermore wait until a toluene separated. Proline absorbance was measured at λ 520 nM. The content of proline is calculated based on a standard curve of proline.

Percent of the cumulative effect of a multiple stress is the correlation between changes caused by the multiple stress with changes caused by the single stress cause greatest changes in the level of equal treatment of the parameters measured. Determination percent cumulative effects of multiple stresses are determined based on the Alexieva et al. formula.[10]

Quantitative data obtained from this research was analyzed using analysis of variant (Anova) to determine the effect of single treatment and the correlation between treatments toward measured parameters. In addition, Duncan's Multiple Range Test (DMRT) was used to determine the significant differences between treatments at 95% confidence level.

3. RESULTS AND DISCUSSION

Results of the research showed that there was an interaction between purple nutsedge interference and drought stress treatments on proline accumulations in soybean leaves. Proline accumulation in soybean leaves due to purple nutsedge interference and drought are shown in Table I. Soybean plants without drought or with mild drought stress, the presence of three or six purple nutsedge interference did not affec on the proline accumulation. In soybean plants with severe drought stress, an increase interference from three to six purple nutsedge showed increased proline accumulation. These conditions indicates that the treatment of the same drought stress, increased purple nutsedge interference lead to increased drought stress that occurred on purple nutsedge and soybean crop. This is in accordance of Morvillo et al. opinion,[2] that the adverse effects of weeds on crop directly through alellopathy and competition for limited resources such as water.

Table I. Everage of proline acummulation (μmol/g) in soybean (*Glycine max* L.) leaves due multiple stress of purple nutsedge (*Cyperus rotundus* L.) interferences and drought.

	Content of purple nutsedge			
Drought	0	3	6	Everage
Control	0,11^{d}	0,11^{d}	0,10^{d}	0,11
Mild	0,13^{d}	0,13^{d}	0,15^{d}	0,13
Severe	1,86^{c}	1,99^{b}	2,28^{a}	2,04
Everage	0,70	0,74	0,84	(+)

Notes: The numbers followed by different letters indicate a significant difference with duncan multiple test at level of 95%, each treatment with 5 replications.

Drought stress was defined as a decreased of the availability of groundwater level that can be quantified as soil water potential. In these conditions, the soil water potential may be lower than the plant tissue water potential of so that it becomes more difficult absorbs water.[11] This will trigger the plant to synthesize and accumulate organic osmolitikum include proline in response to drought stress. Biosynthesis of proline occurs in the cytosol of glutamate were reduced to glutamate seminaldehyde (GSA) by the enzyme pyrroline-5-carboxylate synthetase (P5CS), then GSA is converted spontaneously into pyrroline-5-carboxylate (P5C) further P5C reduced to proline by enzyme P5C reductase (P5CR). P5CS gene activity triggered by ROS and AS signal.[18] Increased of proline content were caused by the drought stress also occurs on *Helianthus annuus* L. Hybrids[20] and eight upland rice varieties namely var. Becon, Kusam, Nabawan, Bertin, Pulot wangi, Hirta, Tenon and Sintok.[21]

Proline accumulation that occurs in plants that undergo stress can lower water potential tissue becomes lower than the potential groundwater without disturbing the metabolism of plants,[19] thus allowing the plant roots can absorb water in drought stress conditions. In addition to the above functions proline, proline can act as an antioxidant by reacting with some form of ROS which accumulate in large quantities as a result of stress and produce compounds that are stable. Proline reacts with OH (radical hydroxy) produces hidroksiprolin, then will react with H_2O_2 to produce the radical proline nitroksil and react with $^1O_2$ produce $^3O_2$, but the reaction with H_2O_2 is very slow, the dominant adsorb ROS reaction proline with $^1O_2$.[14]

The results of the cumulative effect calculation of a multiple stress are presented in Figure 1. Treatment of three purple nutsedge interference in mild or severe drought stress conditions indicates a value below 100% or the character is cross adaptative, which means that the views of the proline accumulation shows that one of the two stress in multiple stress effected reducing soybean plants sensitivity against other stresses. While treatment six purple nutsedge interference on of mild or severe drought

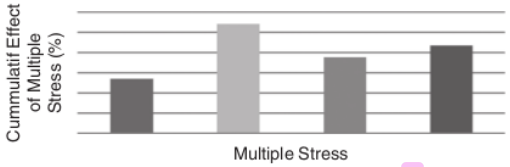

Fig. 1. Percent (%) of cumulative effect multiple stress of purple nutsedge (*Cyperus rotundus* L) interference and drought on proline accumulation of soybean (*Glycine max* L.) leaves.

stress condition indicates a value more than 100% or the character is cross synergism, which means that the views of the of proline accumulation shows that one of the two stress in multiple stress effected increasing of soybean plants sensitivity to another stress.[10] The cumulative effect with cross synergism character also occurs in multiple stresses of biotic and abiotic to the decline in soybean shoot growth.[22]

4. CONCLUSION

From the results of this study can be concluded that an increase of proline accumulation in soybean as tolerance mechanism againts of severe drought stress in the presence of three or six purple nutsedge interference. The cumulative effect character of multiple stresses three purple nutsedge interference in mild or severe stress condition is cross adaptative, while the six purple nutsedge interference in mild and severe drought stress is cross synergism.

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