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The Impact of Water Deficit on The Soybean (*Glycine max* L.) Reproductive Stage of Development

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ABSTRACT

Experiment was conducted in 2014, 2015 and 2016 at the Institute of Field and Vegetable Crops, Novi Sad, Serbia, to study the effects of water deficit treatments during a soybean reproductive stage. Two water deficit treatments were conducted T_1 (from beginning of blossom to seed maturation) and T_2 (from beginning of seed filling to seed maturation period) and control (no water deficit). The results showed that water deficit during seed formation has a significant effect on germination energy, germination precentage and abnormal seedling. Obtained results are of great importance for farmers, because under favorable conditions, a large number of abnormal seedling has the ability to sprout, reach the reproductive stage and participate in yield formation.

Key words: Germination, *Glycine max* L., Reproductive stage, Water deficit.

INTRODUCTION

Water is considered as one of the most important environmental factors, that reduces crop productivity more than any other factor (Lambers *et al*., 2008). Plant response on water deficiency is very complex trait that includes genetic, morphological, physiological and biochemical mechanism (Saxena *et al*., 2019). Soybean is very sensitive to water deficit at certain stages of development and impact of water deficit depends on its length and intensity (Shadakshari *et al*., 2014). Magdi *et al.* (2017) found that the water deficit during blossom stage and the beginning of seed formation significantly increased the rate of abortion of pods. Water deficit during the late reproductive stage affects the accelerated leaf aging, (Sionit and Kramer, 1977) which leads to a decrease in yield (Núñez *et al*., 2005).

From all of the above it can be seen that the authors mainly examined impact of water deficit at certain stages of development. However, in production stage water deficit is not only related to one physiological stage of plant development. Therefore, the study of water deficit impact during a longer period of time (in stages where soybean in field conditions are most often exposed to water deficit) on quality of the formed seed, has significance for agricultural producers.

MATERIALS AND METHODS

Weather conditions

Experiment was conducted in 2014, 2015 and 2016 in the semi-controlled conditions, at the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The average daily temperature during experiment did not differ significantly in three years of the study. In 2014, the mean decadal temperature was 20.09°C, in 2015, 21.76°C and in 2016, 20.98°C. Data was taken from Republic Hydro-meteorological Service of Serbia.

Plant materials

Experiment was set up in Mitcherlich pots (a total of 105 pots, with 5 plants per pot) by random block system in three replications. All plants were under the same irrigation layout (80% FWC) until the beginning of flowering. Plants from 35 pots were exposed to water deficit (60-65% FWC) from the beginning of flowering (R1 soybean reproductive stage) until the end of the maturation period (T_1). Plants from other 35 pots were exposed to the soil drought of the same intensity (60-65% FWC) from the beginning of the seed filling (R5 soybean reproductive stage) until the end of the maturation period (T_2). Plants from the remaining 35 pots were optimally (80% FWC) provided with water during the whole reproductive period (Control). During the vegetative period, in all years of research, on soybean plants have not been identified diseases, nor insect damage. Harvesting was done manually and then four average samples were formed for each treatment.

Germination energy and germination was tested using standard germination test and the cold test (ISTA, 1996). After eight days of germination, in soybean seedlings that germinated under optimal conditions (using standard germination test) was determined the intensity of lipid peroxidation was determined by the method of Placer *et al*. (1966), concentration of free proline by Bates (1973) method and vitamin C by Benderitter *et al*. (1998). Protein and oil content was determined by the NMR method (nuclear

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magnetic resonance) according to Granlund and Zimmerman (1975).

Statistical analysis

Data analysis was done using the statistical software package 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA). Obtained results were processed by method of three-way analysis of variance. Individual testing was carried out using Tukey's for the probability p≤ 0.05. The standard error of the arithmetic mean was used to calculate the significance of the difference using the Tuckey's test.The results presented in the graphs were obtained on the basis of one-way analysis of variance. The interval bars in the figures represent the difference between the standard error of the arithmetic mean.

RESULTS AND DISCUSSION

Germination energy, germination, abnormal seedlings

The water deficit in the reproductive stage had a negative impact on seed quality. The decrease in quality depended on the length of the water deficit. Under optimal temperature conditions, germination energy was 15% lower at T_1 and 9% at T_2, while germination was 9% and 6% lower, respectively (Fig 1, 2). The negative effect of water deficiency is more pronounced, but without statistical significance, on germination in the case when the seeds are sown after harvest under stressful conditions or in conditions of lower temperature (Fig 3, 4). There are contradictory results when it comes to the impact of water deficiency on seed germination. According to some scientists, the water deficit in the seed formation stage has a negative effect on its germination and according to other scientists it does not. Heatherly (1993), on the example of soybeans, found that a water deficiency influences on a decrease in seed quality, regardless of what period of the reproductive stage the plant was not sufficiently provided with water. Dornbos *et al*. (1989) found that with increasing water deficit, the period from the beginning of intensive seed filling and physiological maturity decreased from 17 to 27%, with seed germination decreasing by 6%. Smiciklas *et al*. (1989) state that seeds exposed to water deficit at the germination stage had 10% lower germination (86%) compared to seeds that developed under conditions of optimal plant water supply (germination rate of 96%). Contrary to these experiments, Vieira *et al*. (1992) found that lack of water during seed formation does not affect the decrease in its germination. Also, the results showed that the water deficit had a great influence on the content of abnormal seedling. Under optimal temperature conditions, the content of abnormal seedling was 9% higher at T_1 and 5% higher than the control at T_2 (Fig 5, 6). Under stress conditions, only the T_2 variant showed a difference in the number of abnormal seedling, 3%. Dead seeds were statistically significantly higher during germination under stress conditions, 11% and 4% respectively higher than controls. Abnormal seedlings are not counted as germinating seeds in the standard germination test, as it is assumed

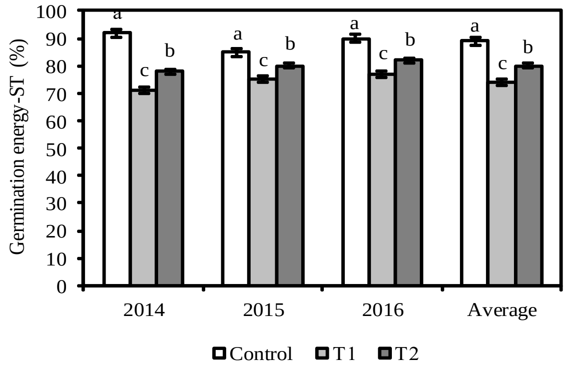

Fig 1: The effect of water deficit on germination energy (standard test).

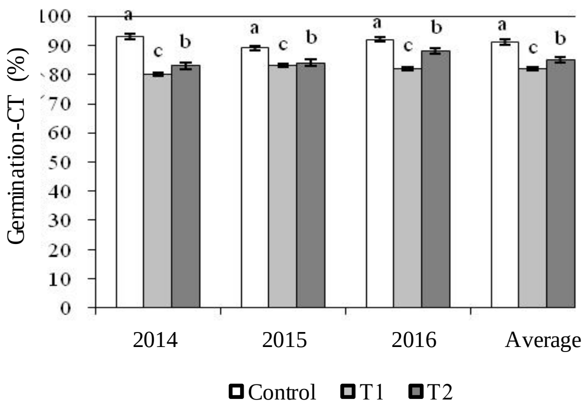

Fig 2: The effect of water deficit on germination energy (cold test).

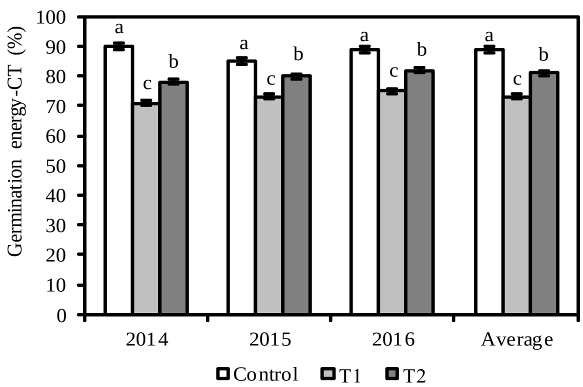

Fig 3: The effect of water deficit on germination (standard test).

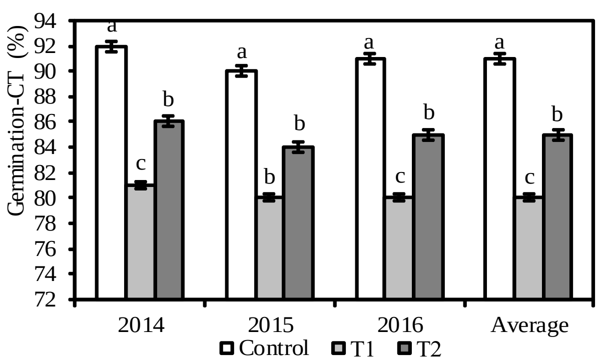

Fig 4: The effect of water deficit on germination (cold test).

that they will not yield a normal plant. However, according to experiments conducted on maize in favorable conditions, many abnormal seedlings have the ability to sprout, reach the reproductive stage and participate in yield formation (Vujošević *et al*., 2018). Seeds formed under water deficiency also have a high proportion of abnormal seedlings, if germination is performed under optimal germination conditions. However, if germination is carried out at low temperatures then a higher proportion of dead seeds is significantly higher than abnormal ones. The results are of great importance for farmers as they show that soybean seeds formed under water deficiency have the ability to germinate. However, with seeds like this there is a big difference between germination energy and seed germination. The reason is that such seeds need more time to absorb water and imbibition, resulting in slow seed germination.

Biochemical parameters in seedlings

With the increase in the length of the water deficit, there was an increase in the intensity of lipid peroxidation, the content of free proline and vitamin C in soybean seeds. The T_1 variant had an increase of 15.74% and the T_2 variant of 8.13% higher intensity of lipid peroxidation, while the free proline content increased by about three and two times, respectively, in soybean seedlings compared to the control (Fig 7, 8). A similar conclusion was reached by Li *et al.* (2013) on the seeds of the plant *Eremosparton songoricum* (Litv.) Vass. They point out that a greater water deficit results in a more intense accumulation of MDA which results in damage to the cell membranes, resulting in a decrease in seed quality. A large body of data indicates a positive correlation between proline accumulation and plant stress caused by the action of one of the environmental factors. Free proline is an amino acid that plays a very important role in plants exposed to different stress conditions. In addition to acting as an excellent osmolyte, free proline plays three major roles during stress, ie. as a metal chelator, an antioxidant defense molecule and a signaling molecule (Hayat *et al*., 2012). Also, due to the effect of unfavorable environmental factors, a higher amount of vitamin C accumulates. A significantly higher amount of vitamin C was accumulated in seedlings from the T_1 variant, 50.90%, while in the T_2 variant, it was 25.65% more accumulated compared to the control (Fig 9). Tuteja *et al*. (2012) point out that an increase in vitamin C content is of great importance, especially in the case of legumes, which are very sensitive to the adverse influence of environmental factors. Vitamin C under unfavorable conditions has the ability to activate a complex of defense mechanisms and thus to reduce the negative effect of abiotic factors (Conklin, 2001).

Chemical composition of seeds

The protein and oil content of the seed depends primarily on the genetic factors, but also on the effect of environmental factors (Wilcox *et al*., 1985). This is confirmed by the results of the soybean research. The water deficit from the beginning

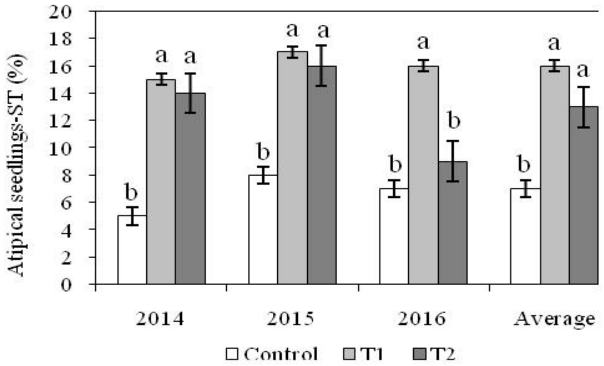

Fig 5: The effect of water deficit on atypical seedlings (standard test).

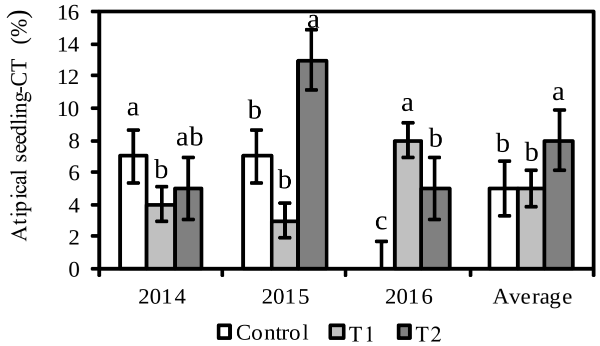

Fig 6: The effect of water deficit on atypical seedlings (cold test).

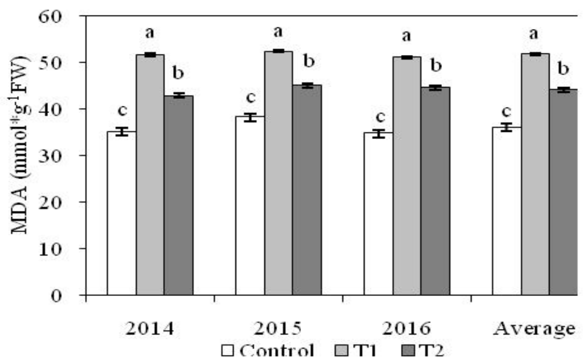

Fig 7: The effect of water deficit on MDA content.

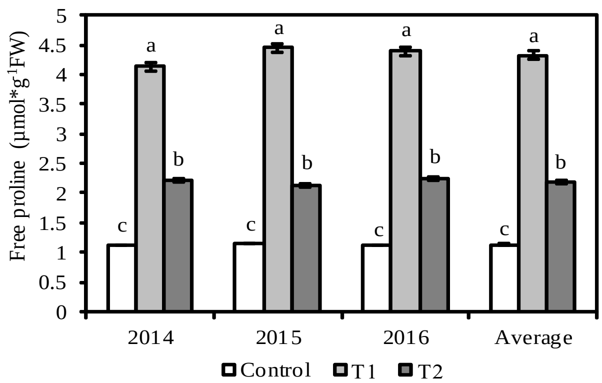

Fig 8: The effect of water deficit on content of free proline.

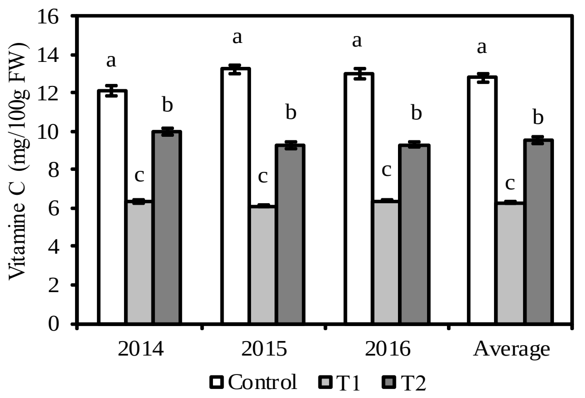

Fig 9: The effect of water deficit on content of vitamin C.

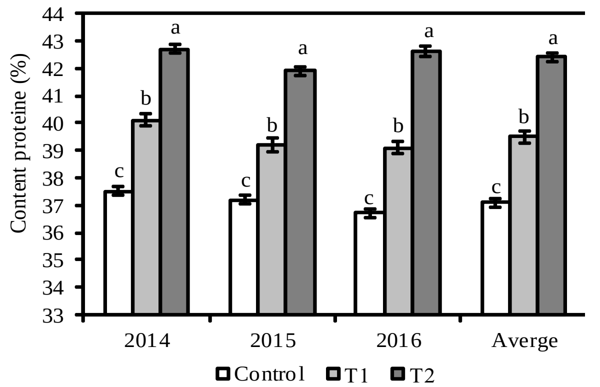

Fig 10: The effect of water deficit on the content of protein in soybean seed.

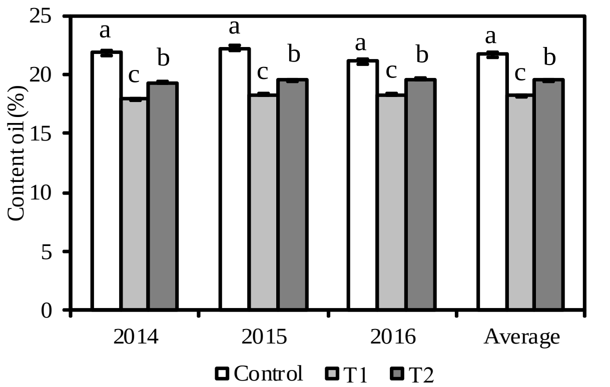

Fig 11: The effect of water deficit on oil content in soybean seed.

of flowering to the seed maturation stage had a greater effect on increasing the protein content than on reducing the oil content. Protein content was 42.4% in T_1, 39.5% in T_2 and 18.2% and 19.5%, respectively (Fig 10). Experiments have shown that plants exposed to the water deficit from the beginning of flowering to the end of the maturation period had a 5.3% higher protein content than plants that were optimally provided with water. The water deficit from the beginning of seed filling to the end of the maturation period increased the protein content by 2.9%. The results are in agreement with the research conducted by Rotundo and Vestgate (2010). They found that the protein content increased by 2 to 23%, depending on the length of the water deficit, relative to plants that were optimally provided with water. A similar conclusion was reached by Carrera *et al.* (2009). They point out that the protein content of soybean seeds, in addition to the length of the water deficit, also depends on the intensity of the water deficit. Lemaitre *et al.* (2008) found that, if there is a water deficit, the plant to a lesser extent absorbs nitrogen in the form of nitrate from the soil solution and for the most part it mobilizes from other plant parts to the seed as well as to the young leaves. Ozturk and Aydin (2004) found that the increase in protein content due to water deficit is mainly due to higher accumulation of nitrogen in the seed and lower accumulation of carbohydrates. Water deficit also led to a decrease in oil content. Plants exposed to the water deficit from the beginning of flowering to the end of the maturation period had a 3.2% lower oil content compared to plants that were optimally provided with water (Fig 11). Smiciklas *et al.* (1989) point out that the water deficit in the grain filling phase has the greatest influence on reducing the oil content.

CONCLUSION

Based on three years of research into the impact of the water deficit in the reproductive stage of soybean development, the following conclusions can be drawn:

• Seed quality is deteriorating and significant deterioration is present in seeds that are sown after harvest under stressful conditions or in the germination phase exposed to a lower temperature.

• Protein content increased while oil content decreased in water deficit conditions during the generative period. As the length of the water deficit increases, the effect is more pronounced.

• The intensity of lipid peroxidation, the content of free proline and vitamin C were increased in seedlings that originated from seeds formed under conditions of water deficit.

ACKNOWLEDGEMENT

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