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Effect of seed priming using KCl on the growth and proline accumulation of paprika (*Capsicum annuum*) growing at different water availability

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Abstract. *Solichatun, Putri TA, Mudyantini W, Pitoyo A. 2022. Effect of seed priming using KCl on the growth and proline accumulation of paprika (*Capsicum annuum*) growing at different water availability. Asian J Trop Biotechnol 19: 1-6.* Paprika (*Capsicum annuum* L.) is a vegetable commodity that has high economic value. Paprika cultivation still faces problems related to drought conditions. One technique to increase plant resistance to drought stress is the seed priming technique. Seed priming treatment can use various solutions, including KCl. The purpose of this study was to determine the effect of seed priming with KCl solution on the growth of paprika and accumulation of proline. This research is experimental, using a completely randomized design (CRD) with a combination of seed priming treatment and water availability. Seed priming treatment was variation of concentration of KCl solution of 10, 20, and 40 ppm. The treatment of variations in water availability was 100% field capacity (FC), 75% FC and 50% FC. The experiment used 3 replications. Seed priming treatment using KCL at various concentrations (10, 20, and 40 ppm) significantly affected the growth of paprika (*C. annuum*) that grow on variations in water availability. Seed priming treatment using KCl can increase the resistance of paprika plants to moderate drought conditions. Paprika resistance to drought is characterized by accumulation of proline and regulation of root to shoot ratio.

Keywords: KCl, paprika, proline, seed priming, water availability

INTRODUCTION

Paprika (*Capsicum annuum* L.) is one of the chili varieties grown in Indonesia. In general, the success of paprika and chili cultivation is still constrained by several factors. These factors include the presence of disease and environmental stress. Water availability is one of the environmental factors that cause stress for plants. Water stress occurs when the availability of water in the environment is not at an optimal level. Water stress can be in the form of excess water (flooding) or lack of water (drought).

Drought is considered as one of the most destructive abiotic stresses across the world and creates a huge impact on crop production (Marthandan et al. 2020; Zhu et al. 2021). Drought is an environmental condition that very often inhibits chili growth. Drought stress causes low productivity (Nawiri et al. 2017; Begna 2020). The long dry season is one of the causes of drought in agricultural land. Drought stress can have a negative impact on chili growth. Reducing water availability can cause vegetative growth such as plant height, root length, root dry weight, plant dry weight decreases. The low availability of water also causes the generative growth of chili to be inhibited. The results of the research by Yusniwati et al. (2008) showed that drought stress treatment on several varieties of chili can reduce chili production (Tit Super 29.2%, Jati Profit 47.72%, Hot Chilli 25.74%, Laris 52.63%, and Prabu 50.83 %). According to Matiu et al. (2017), globally, drought has reduced the production of several other horticultural crops, namely corn (11.6%), wheat (9.2%), and soybeans (33.1-12.2%). Drought stress can affect plant growth and production.

Physiologically, plant tolerance to drought is carried out by accumulating proline compounds that act as osmoregulatory and osmoprotectant compounds. Under drought stress conditions, there was an increase in the percentage of leaf proline content compared to normal conditions. Based on the research results of Yusniwati et al. (2008) the leaf proline content in some chili genotypes increased by 12.62% to 646.31%. Drought conditions can also cause metabolic activity disorders in plants due to a decrease in chlorophyll content. Based on research conducted by Armita et al. (2017), the content of chlorophyll a, chlorophyll b and total leaf chlorophyll of control plants were higher than plants that grew under drought stress conditions.

The growth and development of seedlings are one of important stages in plant cultivation. The growth and development of seedlings determine growth at later stages in the plant life cycle. The problem faced in the growth phase of cayenne pepper seedlings is the very fast vegetative growth of the aerial parts. According to de Rezende et al. (2017), growth imbalance results in the formation of fragile and elongated seedlings, with smaller hypocotyl diameters, producing fewer roots, and having a higher sensitivity to biotic and abiotic stresses. These factors can cause a decrease in the ability to grow and develop seedlings. Uniform seed germination and good and healthy seedling development are key factors for increasing agricultural production.

Seed priming is an alternative technology to overcome seed quality by giving certain treatments to seeds before planting. Seed priming is defined as the preparation of seeds using a variety of procedures in order to increase seed germination rate, percentage germination, and seedling emergence uniformity by manipulating the amount of water available in the seed. The pretreatment starts the germination process but prevents radicle protrusion, after which the seeds are dried until needed. Seed priming treatment can use various solutions, namely polyethylene glycol (PEG), mannitol, sorbitol, glycerol, organic salts such as NaCl, KCl, KNO_3, $MgSO_4$, $CaCl_2$, vermiculite, activated charcoal, clay; or hormones and growth regulators (PGR) such as paclobutrazol, abscisic acid, IAA, gibberellins, kinetin, polyamines and salicylic acid (Mirmazloum et al. 2020).

Plant tolerance to drought stress is controlled genetically and is expressed phenotypically through morphological and physiological adaptations. Based on the research of Naz et al. (2014), KCl treatment in *Pisum sativum* L. seeds can increase root elongation. The treatment of soaking seeds in 2.5% (w/v) KCl solution can induce drought resistance in wheat seeds (*Triticum aestivum* L.) (Eivazi 2012). Considering the economic importance of paprika and also for the development of its cultivation techniques, research on seed priming using KCl on paprika seeds needs to be done. This study aims to determine the effect of seed priming using KCl on the growth and accumulation of proline paprika (*C. annuum*) grown in various water availability.

MATERIALS AND METHODS

Paprika seeds used were taken from ripe fruit. The fruit was obtained from paprika farmers in the Cepogo area, Boyolali, Central Java, Indonesia, harvest year 2020. Growth and germination tests were carried out at the Biology Laboratory of Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret (UNS), and the greenhouse of *Unit Pelaksana Teknis* (UPT) Central Laboratory of UNS, Surakarta, Central Java, Indonesia.

The materials used for seed priming are KCl and aquades. KCl was dissolved in distilled water according to the concentration variations of 10, 20, and 40 ppm. The materials used for the seed viability test were 2,3,5-triphenyltetrazoliumchloride, KH_2PO_4, $Na_2HPO_4.2H_2O$, and aquades. The media used for the growth test was regosol soil that was given compost with a ratio of 2:1. Chemicals to determine proline levels are standard proline, 3% sulfosalicylic acid, ninhydrin acid, toluene, and glacial acetic acid (AAG).

Seed preparation

The seeds are removed from the paprika fruits, then air-dried for 1 week. Dried seeds were used for testing. The seeds used are selected in good condition and uniform (shape, size and color).

Seed viability test

Seed viability assay was done by tetrazolium-based method. For tetrazolium staining, 100 seeds were put in a wet paper at 20ºC for 18 hours. Seeds were then wounded by cutting the testa between the radicle and the cotyledon. Wounded seeds were immersed in 1% tetrazolium chloride solution (in phosphate buffer) for 6 hours at 30ºC, in dark conditions (ISTA 2016). Seeds were cut in half, observed, and counted the viability percentage based on red color of seed.

The percentage of seed viability is calculated by the following formula:

$$\text{Seed viability (\%)} = \frac{\text{number of viable seeds x 100\%}}{\text{total number of seeds}}$$

Seed priming treatment using KCl solution at various concentrations

KCl solution was applied as seed priming at concentrations of 10 ppm, 20 ppm and 40 ppm. Paprika seeds are soaked in the solution for 24 hours. After that, the seeds are drained and air-dried at room temperature for 24 hours. The dried seeds will be used for the growth test.

The growth test using different water availability

The water availability test was carried out in polybags filled with soil. The size of the polybag used is 10 x 20 cm. The soil used was regosol type with a pH of 6.0. Each polybag is filled 1000 gram regosol soil. Three seeds were sown per polybag at the depth of 3 cm. The polybags were placed in the greenhouse. The research design used was complete randomized design with 5 replications. The treatment under study is as follows :

100% field capacity = 500 mL of water volume
75% field capacity = 375 mL of water volume
50% field capacity = 250 mL of water volume

Determination of the field capacity of the soil using the gravimetric method. One kilogram of wind-dried soil is weighed and put into a polybag that has been provided with drainage holes. Water is sprinkled on the ground, and left until the soil is evenly wet and water drips from the drainage hole. The soil is left for 6 hours (until the water is no longer dripping). This condition is a condition of 100% soil capacity. The soil is then weighed again, and the difference between its weight and the initial weight of the soil (dry soil) is calculated. The figure obtained is the volume of water that must be added to reach the specified field capacity. The water content of the media is checked every day, and given water so that it is at the specified water content. The data for the plant height, leaf area, fresh weight, root to shoot ratio (fresh weight of root biomass divided by fresh weight of shoot biomass), chlorophyll content, carotenoid content and also proline content was recorded after 2 months of sowing (Kurniawan et al. 2010; Sayyari and Ghanbari 2012; Gayathri et al. (2016).

Shoot: root ratio determined based on fresh weight, according to Kakanga et al. (2017). Chlorophyll and carotenoid content were determined using spectrophotometer (Hendry and Grime 1993). Leaf proline levels were calculated at the end of the growing season using spectrophotometer according to Bates et al. (1973). Samples of fresh leaves (2nd leaf from the tip of the plant) weighing 0.1 g were cleaned and crushed, then extracted with 5 mL of 3% sulfosalicylic acid. After homogenization, 2 mL of the sample was taken and added with 2 mL of 0.14 M ninhydrin acid and 2 mL of glacial acetic acid (AAG) then water bathed at 100^{o}C for 1 hour. The tube containing the sample was put into a beaker containing ice cubes for 5 minutes. The sample was extracted again with 4 mL of toluene and shaken for 15-20 seconds. After shaking it will form 2 layers of liquid. The pink layer was taken and the absorbance was calculated by means of a UV-visible spectrophotometer at a wavelength of 520 nm. The standard solution used is L-proline. There are 5 levels of proline concentration as a standard solution, namely 0.1 M, 0.2 M, 0.3 M, 0.4 M and 0.5 M, and is calculated based on the standard curve.

Data analysis

Quantitative data were analyzed by ANOVA (Analysis of Variance) and if there was a significant difference between the treatment groups, further tests were carried out using Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

RESULTS AND DISCUSSION

Seed viability test

The tetrazolium test was carried out at the beginning of the study to determine the viability of the seeds to be used. The results of the tetrazolium test showed that the seeds used had a viability of 100% (Figure 1).

Growth parameters

Growth parameters observed in this study were plant height, leaf area, fresh weight, and shoot:root ratio, presented in Table 1. The results of the analysis of variance showed that combination of seed priming treatment using KCl and water availability had a significant effect on the height of paprika seedlings (Table 1).

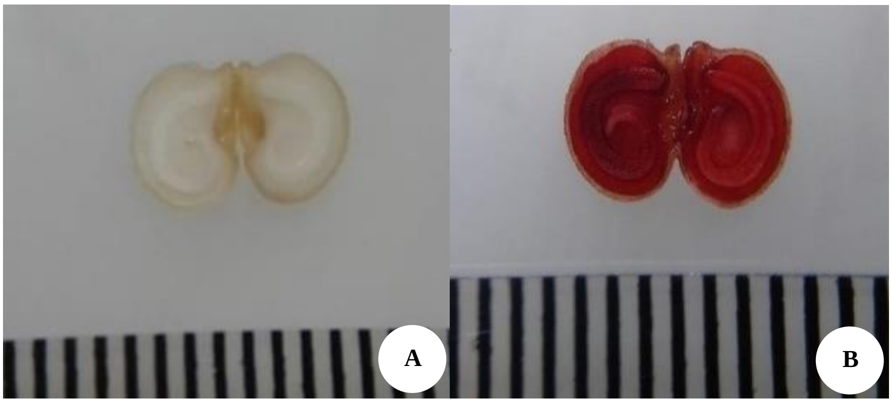

Figure 1. A cross-section of paprika (*Capsicum annuum*) seeds. A. Before soaking pepper seeds in 1% tetrazolium solution, B. After paprika seeds soaking in 1% tetrazolium solution

Table 1. Growth of paprika (*C. annuum*) seedling after seed priming and water availability treatments

Combination treatment (KCL concentration and water availability)		Plant height (cm)	Leaf area (cm^2)	Fresh weight (gram)	Root to shoot ratio
Control (without priming)	50 FC	5.0^{ab}	2.39^{ab}	0.53^{ab}	1.00^{a}
	75 FC	8.6^{bcd}	3.25^{bc}	0.52^{ab}	2.32^{abc}
	100 FC	4.0^{a}	1.62^{a}	0.46^{a}	0.93^{a}
KCl 10 ppm	50 FC	5.3^{ab}	2.69^{ab}	0.38^{a}	1.37^{ab}
	75 FC	6.6^{abc}	2.21^{ab}	0.74^{ab}	1.78^{ab}
	100 FC	6.5^{abc}	2.30^{ab}	0.56^{ab}	1.75^{ab}
KCl 20 ppm	50 FC	6.5^{ab}	2.52^{ab}	0.37^{a}	1.64^{ab}
	75 FC	9.7^{cd}	2.67^{ab}	0.74^{ab}	1.29^{ab}
	100 FC	11.5^{de}	3.26^{bc}	1.11^{b}	2.23^{abc}
KCl 40 ppm	50 FC	11.1^{de}	3.40^{bc}	0.48^{a}	2.57^{ab}
	75 FC	14.9^{e}	3.28^{bc}	2.43^{c}	3.42^{c}
	100 FC	8.7^{bcd}	4.00^{c}	0.32^{a}	1.88^{ab}

Note: Different letter notation in the same column shows significantly different using Duncan's Multiple Range Test (DMRT) at a 95% confidence level; FC means field capacity

The plant heights from seeds that were not treated (control) and those that were treated with 10 ppm KCl were not significantly different in all variations of water availability, but treatment using KCl 20 and 40 ppm significantly increased plant height at all levels of water availability. The highest plant height resulted from 40 ppm KCl treatment.

At 50% field capacity water availability, the 40 ppm KCl treatment significantly increased paprika plant height. This means that seed priming treatment using KCl can stimulate paprika plants to grow well at low water availability. According to Putra et al. (2017), 50% level of field capacity similar than medium drought stress, so that seed priming using KCl has the potential to be used to increase the resistance of paprika plants to drought. Figure 2 shows the morphology of paprika seedlings that have been treated with different seed priming and water availability.

Besides the plant height parameters, according to Table 1, the combination of seed priming treatment using KCl and water availability also had a significant effect on the leaf area, fresh weight, and shoot:root ratio of paprika seedlings. The lowest leaf area resulted from control plants growing at 100% water availability; while the highest leaf area was produced from plants treated with 40 ppm seed priming and grew at 100% water availability. These results indicate that seed priming treatment could increase the growth of paprika plants. Higher leaf area can increase the area of light absorption for photosynthesis. The increased rate of photosynthesis causes increased production as well. In the 40 ppm seed priming treatment, the leaf area of paprika plants growing at various levels of water availability was not significantly different. These results indicate that seed priming treatment causes plants to grow well in conditions of low water availability (medium drought stress).

Plants express a dynamic response to sustain under stress conditions through morphological, physiological, and biochemical changes. The response of plants to drought stress conditions has been categorized into drought escape, drought avoidance, and drought tolerance (Marthandan et al. 2020). Determination of root to shoot is one of the adaptations of plants to drought stress. The root to shoot ratio depends upon the partitioning of photosynthate which may be influenced by environmental stimuli. The results of the ANOVA showed that seed priming treatment and water availability had a significant effect on the root to shoot ratio of paprika plants. The highest root to shoot ratio value was obtained from the seed priming treatment of 40 ppm, which grew at 75% water availability of field capacity. This indicates that under these conditions, the shoot biomass is low while the root biomass is high. In drought conditions, plants will increase their root growth in order to optimally absorb water and nutrients from the soil. Changes in the metrics of root-to-shoot relationships can compensate for moisture deficiency and maintain stomatal conductance under drought stress conditions (Kim et al. 2020). Drought stress can cause inhibition of cell division, enlargement and elongation caused by a lack of water availability (Rosawanti 2015). Drought stress can also cause a decrease in plant water potential, thereby reducing cell turgor. Then due to the decrease in cell turgor, it will inhibit the process of cell division and enlargement, so that the plant becomes short (Osakabe et al. 2014).

Biochemical parameters

Drought stress can alter the physiological and biochemical processes in plants. According to Mibei et al. (2017) the alteration processes including chlorophyll and carotenoid content, which is related to photosynthesis. The concentration of carotenoids and chlorophylls provides information about the level of stress experienced by the plant as well as its ability to endure these stresses. Therefore, due to the significant decrease in carotenoids during drought stress, it is evident that drought may lead to reduction in plant productivity. This is mainly by inhibiting growth and photosynthesis, and is one major limiting factor in agriculture worldwide leading to huge reductions in crop yield.

Table 2. Chlorophyll, carotenoid, and proline concentration of paprika (*C. annuum*) seedling after seed priming and water availability treatments

Combination treatment (KCL concentration and water availability)		Chlorophyll (mg/g of fresh weight)	Carotenoid (mg/g of fresh weight)	Proline (mg/g of fresh weight)
Control (without priming)	50 FC	11.84[ab]	0.65[a]	3.00[cd]
	75 FC	13.4[abc]	0.73[ab]	1.26[a]
	100 FC	15.48[c]	0.80[ab]	1.11[a]
10 ppm	50 FC	13.44[abc]	0.71[ab]	2.60[b]
	75 FC	13.22[abc]	0.69[ab]	2.85[bc]
	100 FC	12.36[ab]	0.87[b]	3.00[cd]
20 ppm	50 FC	12.80[ab]	0.69[ab]	3.48[ef]
	75 FC	14.00[bc]	0.74[ab]	3.23[de]
	100 FC	12.61[ab]	0.62[a]	3.58[ef]
40 ppm	50 FC	11.54[a]	0.68a	3.51[ef]
	75 FC	11.50[a]	0.67a	3.50[ef]
	100 FC	13.47[abc]	0.65a	3.73[f]

Note: different letter notation in the same column shows significantly different using Duncan's Multiple Range Test (DMRT) at a 95% confidence level; FC means field capacity

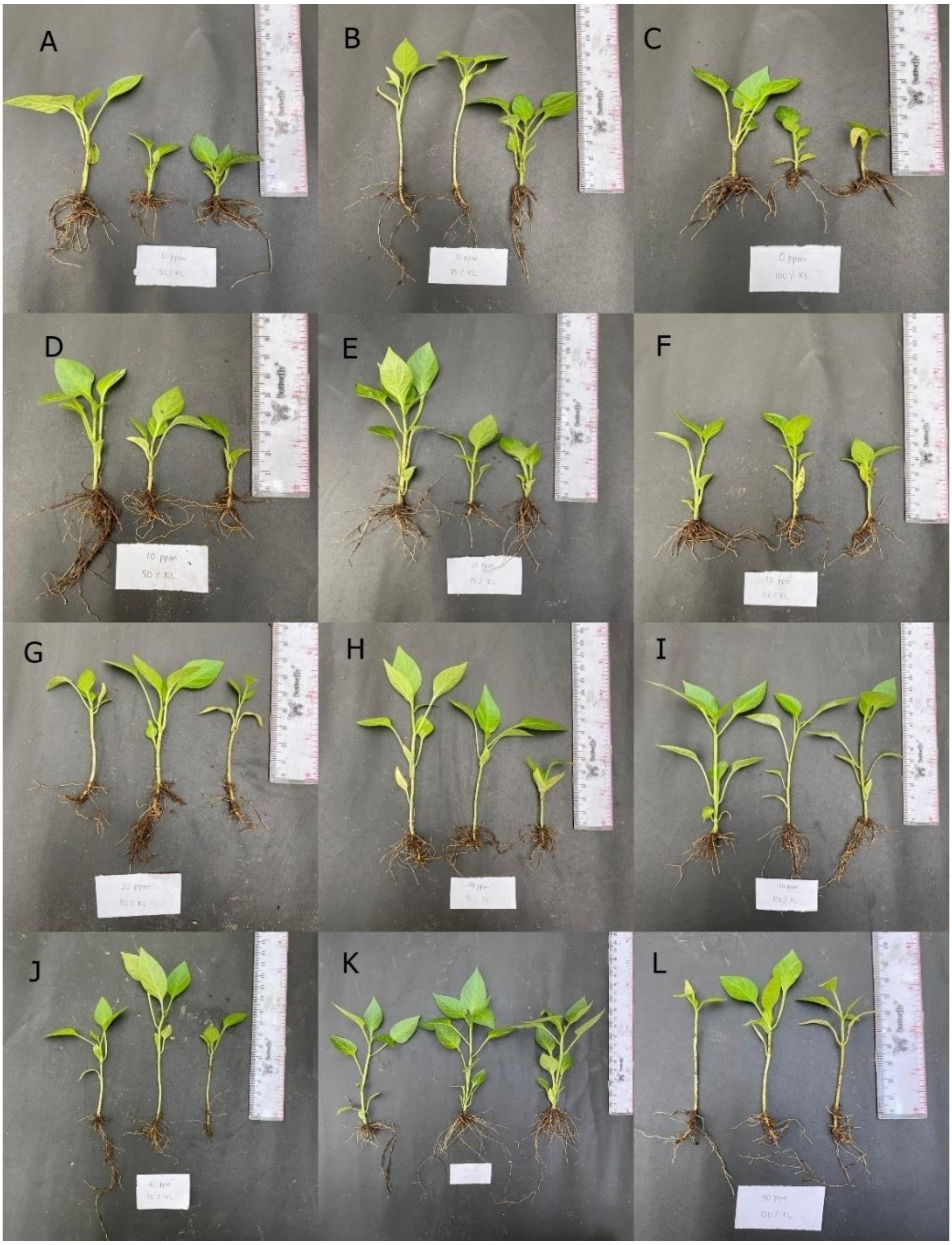

Figure 2. Seedling of paprika (*C. annuum*) two months after seed priming treatment using KCl and grown at different water availability. A: Control + 50%FC; B:control +75%FC; C:control+100%FC; D: 10ppm+50%FC; E:10ppm+75%FC; D: 10ppm+100%FC; G: 20ppm+50%FC; H:20ppm+75%FC; I: 20ppm+100%FC; J: 40ppm+50%FC; K: 40ppm+75%; L: 40ppm+100%FC

In this study, seed priming treatment significantly affected the chlorophyll content of paprika. The highest chlorophyll content resulted from the treatment of 20 ppm KCl at 75% field capacity. Seed priming treatment also significantly affected the carotenoid content of paprika, which is the highest carotenoid content resulting from the treatment of 10 ppm KCl at 100% field capacity. There was no pattern of increasing or decreasing levels of chlorophyll and carotenoids on water availability. According to Rustioni and Bianchi (2021), each plant has a different strategy in dealing with drought stress, for example, drought increases chlorophyll content in stems of *Vitis* interspecific hybrids. The concentration of photosynthetic pigments in woody tissues appeared to be strongly determined by genotypes.

Other metabolic alterations include accumulation of proline induced by drought stress in plants. Proline content of seedlings response to drought stress changed significantly compared to control (Table 1). Proline plays a vital role in maintaining optimal growth in plants under biotic stresses (Kurniawati et al. 2014; Nguyen et al. 2020). The significantly enhanced proline content in paprika leaf in drought stress is a response characteristic of plants like as osmotic adjustor under abiotic stresses. Proline promotes higher resistance in plant cells under adverse environmental conditions. In this study, seed priming treatment significantly affected the proline content of paprika. The highest proline content resulted from the treatment of 40 ppm KCl.

In conclusion, seed priming treatment using KCL at various concentration (10, 20, and 40 ppm) significantly affected the growth of paprika (*C. annuum*) that grow on variations in water availability. Seed priming treatment using KCl can increase the resistance of paprika plants to moderate drought conditions. Paprika resistance to drought is characterized by accumulation of proline and regulation of root to shoot ratio.

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