

Drought Resistance Variation of Mutant of Kenaf KR11 Based on Proline Accumulation

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Accepted 05 March 2014, revised 01 April 2014

ABSTRACT

Kenaf is a fiber plants of the family Malvaceae. KR 11 is a high yielding kenaf variety which is resistant to drought stress. Mutations by EMS at several species known to increase resistance to drought stress by accumulation of osmolite. In this research, physiological testing on 5 mutants to determine the effect of the mutation on the drought stress response of kenaf KR 11 was conducted. Experiment were done by comparing the physiological accumulation of proline in normal and drought stress conditions. The plants were watered every 3 days for 14 days with 100% of field capacity as normal conditions and 25% field capacity as drought stress. The results showed differences in the pattern of proline accumulation between control and mutant in normal and drought stress condition. While control KR11 increased the accumulation on drought condition, mutants showed decreased proline content under conditions of stress with varying decreasing levels. The most significant pattern of proline accumulation compared with control, is shown by the mutant 4 and 5. Proline accumulation pattern is controlled by the proline metabolism control genes, P5CS and ProDH and its feedback regulation. Alteration of this pattern is most likely an effect of mutation induction on the mutant sample.

Keywords : Kenaf, drought, mutant, proline accumulation

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a fiber plant native to East-central Africa where it has been grown for several thousand years for food and fiber. It is a common wild plant of tropical and subtropical Africa and Asia. Kenaf has a unique combination of long bast and short core fibers which makes it suitable for a range of paper and cardboard products [1, 2]. Kenaf has excellent adaptation ability that lead to a good drought stress tolerance. One of the superior kenaf varieties developed by Sweetner and Fibre Plant Research Center, Ministry of Agriculture, in Malang, East Java, is Karangploso 11(KR11). Reference [3] shows that superior characters of KR11 are flooding tolerance, high fibre yield, less sensitive to photoperiodicity, moderate resistant to jassid and tolerant to drought stress.

Completing a variety of superior characters

possessed of KR11, various efforts to increase fiber production can be done. One way is to do a genetic mutation induction. Genetic mutations allows various crop characteristic improvement yet it also allows decline in superior character. Therefore, in applying genetic mutation as plant breeding effort required various observations on the possibility of a change of character. This observation is particularly aimed at the superior characters which are expected to be maintained in line with the improvement of the other characters.

In this study, 5 samples EMS mutant of KR11 Kenaf were observed whether there is any change in the physiological response to drought stress. Physiological responses were occurred to maintain cell's viability and turgidity by inhibiting water release from the cells through osmolyte production. Osmolyte is a compound that serves to maintain the cell turgor pressure in eukaryotes and prokaryotes in the form of organic and inorganic compounds in the cytosol such as proline, sucrose, soluble carbohydrates, glycinebetaine, and various other solutes in cytoplasm to increase the

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absorption of water from the soil [4]. Metabolic adaptation to drought is most easily characterized by accumulation of organic osmolyte such as proline and betaine [5]. Proline is an amino acid that is synthesized through catalysis by two enzymes, namely Δ -1-pyrroline-5-carboxylate-synthetase (P5CS) and Δ -1-pyrroline-5-carboxylate-reductase (P5CR), which uses the cofactor NADPH. P5CS enzyme converts glutamate into proline precursor Δ -1-pyrroline-5-carboxylate (P5C), while P5CR converts P5C into proline. Induced proline production will increase at a variety of stress conditions such as drought, photoperiodicity, salinity and avirulent pathogen attack.

This study aims to understand mutation effect on physiological drought stress response in Kenaf KR11 based on accumulation of proline, which is one of the osmolyte.

METHODS

Plant preparation and stress treatment.

Kenaf seed used in this study were second generation (M1) EMS mutated seeds from previous study of EMS effects on kenaf branching pattern [8]. So, there are one control sample, KR11 and 5 different mutant sample.

Seeds were planted on soil and compost mixture in a pot. On each pot, 3 seedling were grown until watering experiment applied. Seedlings were watered per 2 days until 50 days old. Field water capacity were measured on the 45 days after planting. Watering experiment were performed for two weeks and divided into normal and drought condition. Normal condition was 100% field water capacity volume watering every 3 days. Drought condition was 25% field water capacity volume watering every 3 days. At the end of two weeks treatment, leaves of each plant from the middle part of the stem were collected to measure free proline accumulation content.

Measuring free proline content.

Approximately 0.5 gram of leave was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through Whatman #2 filter paper. Two ml of filtrated was reacted with 2 ml of acid-ninhydrin and 2 ml glacial acetic acid in a test tube for 1

hour in 100°C, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube or using vortex mixer for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for blank. The proline concentration was determined from standart curve and calculated on a fresh weight basis as follows: $[(\mu\text{g proline/ml} \times \text{ml toluene})/115.5 \mu\text{g}/\mu\text{mole}] / [(g \text{ sample})/5] = \mu\text{moles proline / gram of fresh weight material (leaf)}$ [9].

RESULT AND DISCUSSION

Variation in response to drought stress can be determined by comparing the concentration of proline accumulation during treatment given to normal and drought. By measuring the accumulation of proline on the control and mutant samples after watering experiment, there are known differences in the pattern of proline accumulation between control and mutant.

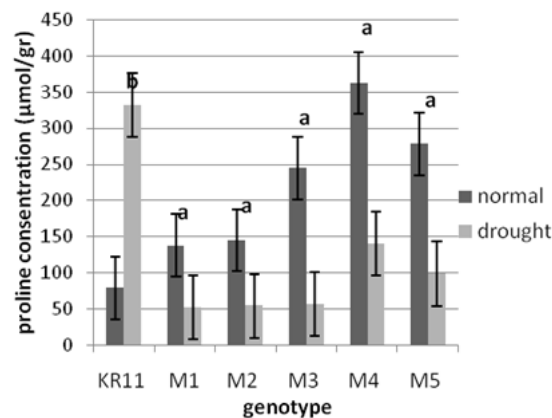


Figure 1. Effect of various EMS mutation induction level on proline accumulation as result of water experiment. Results are shown as mean \pm standard error, obtained from three replicates. Sample followed by the same letter are not significantly different at $P \leq 0.05$, according to Tukey's test.

In plants, drought led to an increased accumulation of proline in plants related to the function of proline as osmolit, osmoprotectan and collecting harmful oxidative compounds (Reactive Oxygen Species, ROS) [10]. Increased accumulation of proline due to genes controlling proline production and expression is

activated in the event of drought stress. On each plant, proline accumulation can vary that may be caused by the natural character of the plant, and the presence of other osmolyte produced. While the differences in the accumulation of proline in each genotype or variety may be affected by the formation of a genotype in which occurs the selection of superior characters and reduction less profitable characters. The formation of this character is also called plant breeding is possible that in a simple cross-breeding, or with more complex through genetic modification. Genetic mutations induced by a mutagen, is one of the breeding effort through genetic modification. Some mutagens are used to work specifically on certain genes that are desired while others are random so as to affect the gene sequences of different genes at the time of use. Mutant genotype in this study is the result of mutations induced by the mutagen ethyl methane sulfonate which is a random mutagen that may cause mutations in various genes.

Different patterns of accumulation of proline in the sample showed the different response of proline accumulation in normal and drought conditions. Accumulation of proline in most non-mutant plants will increase in response to the stress experienced by the plant. In tobacco plants (*Nicotiana glauca*) proline accumulation increased due to salinity stress treatment up to 5 times the normal conditions [7]. Also observed in maize, an increase production of proline in a given sample of water treatment potential drop [8]. Based on both of the published studies, it is known that a decrease in the accumulation of proline in the treatment of drought stress as demonstrated by a mutant samples proline accumulation patterns of unusual or abnormal. This abnormality may be influenced by the induction of mutations in the mutant.

Some of the factors that influence the production of proline include genes that control metabolism of proline and feedback regulatory mechanisms. Reference stated that the various mutations are carried on genes affecting proline biosynthesis and degradation cause changes in the production, accumulation and proline catabolism [9]. There are also feedback inhibition mechanism that affects the biosynthesis and degradation in which the end product and can inhibit the metabolism of labor

between one gene encoding proline metabolic enzymes. In mutant plants, no detectable inhibition feedback mechanism that allows changes in the pattern of accumulation of proline under conditions of stress [6, 9].

Precursors proline production and degradation of proline form is L-glutamic acid. Proline is synthesized from glutamic acid to intermediate Δ^1 -pyrroline-5-carboxylate (P5C) by the enzyme P5C synthetase (P5CS) and P5C reductase (P5CR). Both of these enzymes are synthesized by *P5CS* and *P5CR* gene, and expressed much more in the event of drought stress. While the degradation of proline back to L-glutamate, catalyzed by the enzyme proline dehydrogenase (PRODH) and P5C dehydrogenase (P5CDH) expressed by genes of the same name [5,6]. Degradation of proline done at the time of the re-watering after drought stress and the time of the inundation or flooding stress.

Proline accumulation in drought stress, inhibits the proline degradation process due to *PRODH* gene is hampered by the strong *P5CS* gene expression. Instead in the event of rehydration or flooding, strong expression of *PRODH* gene also inhibits the expression of *P5CS* gene. This causes the ratio of enzymes *P5CS* and *PRODH* and products become influential factors in controlling the level of proline accumulation in cells [6,10].

In general regulatory mechanisms such as the inhibition of the production and degradation that occurs in proline, is influenced by factors that affect such a promoter gene and the gene product. But in proline, it is not known promoter that drives expression of *P5CS* gene and *PRODH* although both expressed strong antagonist of dehydration and rehydration conditions [6]. Based on such information may raise suspicion that the different patterns of accumulation of proline in control and mutant sequences may be affected by changes in genes controlling proline metabolism resulting in a feedback regulation mechanism. For the further analysis to determine differences in the pattern of accumulation associated with mutations in the mutant is the analysis of genetic variation in the gene that controls the metabolism of proline, *P5CS* and *PRODH*.

Mutations that may occur in the gene that expresses both these enzymes EMS-induced mutations can alter the control of production

and catabolism of proline. This resulted in a decrease in plant resistance to stress, especially drought stress. Proline has a variety of roles in overcoming osmotic stress conditions, such as a compatible solute that can keep turgiditas cells, stabilization of proteins, membranes and subcellular structures, and maintain cell function through mechanisms scavenger on Reactive Oxygen Species (ROS). Proline has advantages over other osmolit produced in response to stress that, at high concentrations of proline does not have the effect of bonding between water molecules and macro. So as to keep the subcellular structures from damage under conditions of stress, and maintain turgor pressure so as to maintain cell expansion [11,12].

Mutagen used in the mutant samples, EMS has the ability to change the sequence of DNA through guanine bases changes the molecular structure so it cannot be paired with cytosine with thymine instead. The mutation causes abnormalities in the proteins production so that changes and decreased function or does not operate at all. If attributed to factors and genes that influence the production of proline, it is known that the abnormal accumulation of proline mutants EMS in control and stress conditions may be influenced by the presence of mutations in the genes *PRODH* and *P5CS*. Mutations in the genes that control the production and degradation are possible because the control condition, samples showed a higher accumulation of proline, whereas in the drought stress condition, proline accumulation decreased significantly. This indicates over-expression of the gene *P5CS* experienced under normal conditions (control) and not expressed under conditions of stress drought. As well as also indicated over *PRODH* gene expression during stress drought, so the drastic decrease in the accumulation of proline.

CONCLUSION

Different pattern of proline accumulation in response to drought stress was observed in all samples of different mutant compared to control. This difference may be influenced by mutations in genes that control the production of proline as *P5CS* and *PRODH*. Mutations in

these genes lead to the disruption of both of production and regulation of proline feedback metabolism.

ACKNOWLEDGMENT

The authors gratefully acknowledge the contributions of Plant Physiology, Tissue Culture and Microtechnic Laboratory Staff and fellow researcher for valuable comments on this work and for helping in the real-time simulation

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