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ORIGINAL RESEARCH ARTICLE

CROP IMPROVEMENT THROUGH INDUCING MUTAGENESIS IN VIVO USING COLCHICINE ON COWPEA (*Vigna unguiculata* L. Walp)

I Gede Ketut Susrama* and I Gede Putu Wirawan

Agricultural Technology Department, Faculty of Agriculture, Udayana University
Jl. PB. Sudirman, Denpasar, Bali 80232, Indonesia
**Corresponding author: ketutsusrama@unud.ac.id*

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ABSTRACT

Colchicine treatments of 0.005% and 0.01% either in distilled water or in hydrogen peroxide 3% were caused temporary leaf shape changes on cowpea. Treatment of colchicine in hydrogen peroxide 3% resulted in green pod color change to purple however treatment of just hydrogen peroxide 3% and colchicine in hydrogen peroxide 3% caused 8.8-10.7% dwarf cowpea. As a preliminary data, we also observed that cowpea crops grown from seeds treated with colchicine in distillate water attacked by bean leaf beetle much earlier compare to the treatment with colchicine in hydrogen peroxide 3%.

Keywords: hydrogen peroxide 3%, temporary leaf shape change and pod color change to purple

INTRODUCTION

Mutagenesis have been applied in various fields of research both for development of academic and applied purposes such as genetic modification using mutagen *in vivo* or *in vitro* condition, random or targeted, molecular or non molecular to see cell-shaped changes, number of chromosomes, and or decreasing or increasing stomata numbers per leaf in the aim to improve plant quality. Genetic modification on plant by inducing mutagenesis is potential to improve plant quality considering that the process is relatively simple and does not cost much (Oladosu *et al*., 2016 ; Roychoduwry and Tah, 2016). Parry *et al*., 2009 also mentioned about the important benefit of mutagenesis is to increase flora biodiversity through creating novelty and superior crop cultivar. The discovery of *in vitro* site-directed mutagenesis technique doesn't mean *in vivo* mutagenesis techniques are not needed anymore as Chopra, (2005) mentioned that *in vivo* mutagenesis techniques provide many advantages such as giving possibility to create mutant that having varying degrees of characteristic modification and sequence

analysis of mutant plants will deepen understanding relationship between plant genotype and phenotype.

Induced mutagenesis research started at the time colchicine was invented in year 1940 producing superior plant through chromosome doubling. In an effort to improve plant quality, beneficial mutation that produced through colchicine induced mutagenesis have generated quite a lot of new crop varieties that having better phenotypic characteristics.

MATERIALS AND METHODS

Before the main research we did a preliminary study that was colchicine treatment in distillate water solvent on cowpea seeds. In our main research we did four kind treatments of colchicine on cowpea seeds i.e. 1) cowpea seed treatment with a colchicine solution of 0.005% in distillate water solvent, 2) cowpea seed treatment with a colchicine solution of 0.01% in distillate water solvent, 3) cowpea seed treatment with a colchicine solution of 0.005% in 3% hydrogen peroxide (H_2O_2) solvent and 4) cowpea seed treatment with a colchicine solution of 0.01% in 3% hydrogen peroxide solvent. After 72 hours soaking, all growing sprout were planted in the field. Parameters measured were phenotypic vegetative and generative changes and pests attack.

RESULTS AND DISCUSSION

Preliminary research result

The colchicine treatment on cowpea from preliminary research showed that colchicine in distillate water treatment caused cotyledons enlargement on cowpea seed sprouts which can be considered as a visual evidence that something has happened genetically in this case the possibility of chromosome doubling. There are quite a lot of publicity to do with the treatment of colchicine as chromosome doubling agent that supports above result as Comai (2005) stated that in general polyploid plant having better vigor compare to native varieties. After those sprouts being planted there were leaf shape changed from normal shaped (lanceolate) to heart-shaped (Fig. 1). Those leaves shape changed were in temporary manner, afterward next leaves grow normal, flowering and then producing pods. The effect of colchicine on cowpea apparently was not just on the number of chromosomes. Colchicine in this research look like causing DNA damage resulted in leaf-shaped changed which then can be cured by cowpea DNA repair system.

Fig. 1. Leaf shape change to heart-shaped, stem attaches at the tapering end

Fig. 2. Leaf shape change to heart-shaped and leaf malformation

Colchicine in distillate water treatment

Up to day 11 growth of cowpea grown from seeds treated with colchicine 0.005%, 0.01% and control crops showed almost no difference visually. Leaf-shaped changes from normal leaf-shaped (lanceolate) to heart-shaped and leaf malformation then occurred on crops treated with 0.01% colchicine (Fig. 2). We have found this kind of leaf-shaped changing in our preliminary research as we mentioned above. Leaf-shaped change due to colchicine treatment logically can be happen. Amiri *et al*. (2010) in their publication stated that polyploidy genome could lead to large scale reorganization and subsequently led to various changes in phenotype both in vegetative and generative parts of the plant.

Colchicine in 3% hydrogen peroxide (H_2O_2) treatment

Hydrogen peroxide 3% itself caused leaf malformation and this showed hydrogen peroxide 3% could cause genetic changes on cowpea. As an analog comparison, Ruiz-Laguna and Pueyo (1999) reported that hydrogen peroxide treatment could induces transverse mutation G:C→T:A in *Eschericia coli*. Colchicine in hydrogen peroxide 3% initially slowed the growth of cowpea. Cowpea seed treated with colchicine in 3% hydrogen peroxide solvent compare to control (cowpea seed treated with 3% hydrogen peroxide only) showed very difference growth. At the time control cowpea crops had grown as high as 4-7cm, colchicine treated cowpea has not grown yet. Both colchicine in distillate water and colchicine in 3% hydrogen peroxide caused leaf-shaped changed from lanceolate to heart-shaped. Besides, colchicine in 3% hydrogen peroxide solvent caused other change in leaf shape *viz* from lanceolate to oval (Fig. 3 A).

Those both two kinds of leaf change were temporary.

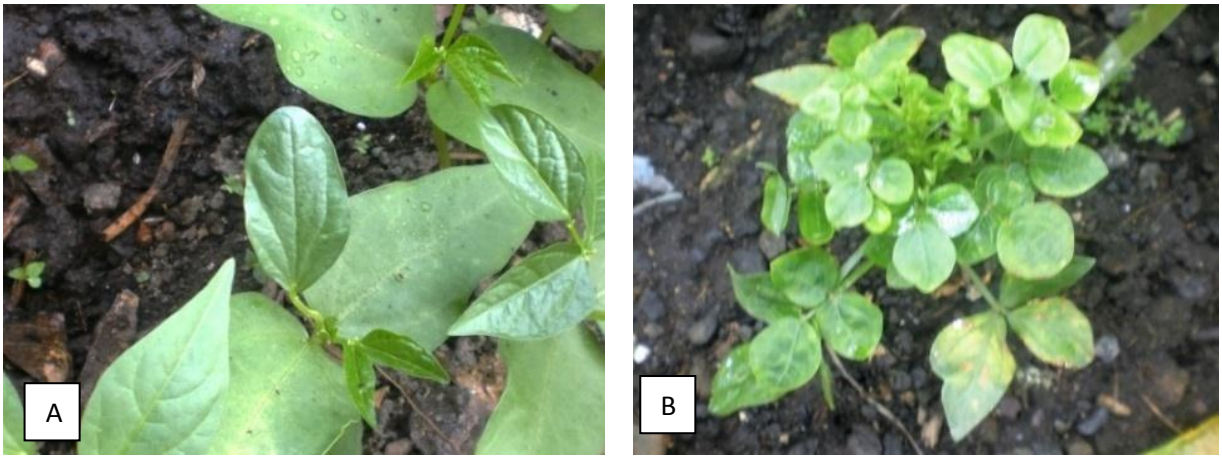

Fig. 3. Leaf-shaped change to oval (A). Dwarf cowpea due to treatment of colchicine in H_2O_2 (B)

Many cowpea crop becoming stunted (Fig. 3 B) due to just 3% hydrogen peroxide treatment and colchicine in 3% hydrogen peroxide. Cowpea with leaf shape change could grow normally however stunted cowpea crop could not grow well meaning that the DNA damage due to colchicine treatment could not be repaired by cowpea DNA repair system. Detail data on the percentage of stunted crops are presented in Table 1.

It is something new in the sense of colchicine which should increase the size of plant with its ability to induced chromosome doubling on plant which expected increasing the size of plant in this research showed the opposite result that caused plant stunting. It is obviously understood from this research that colchicine in distillate water did not caused stunted plant however colchicine in 3% hydrogen peroxide did.

Table 1. Dwarf cowpea due to colchicine treatment in hydrogen peroxide solvent

No	Description	Dwarf Cowpea (%)
1	Control (H_2O_2)	8,8
2	Colchicine 0,005% in H_2O_2	10,0
3	Colchicine 0,01% in H_2O_2	10,7

Pests Attacks

Pests attacks were given a special attention in this research, hoping colchicine treatment giving any initial discovery of mutant cowpea that resistant to certain pest. Soil worm (*Agrotis ipsilon*) which intersecting the base of the cowpea stem underground attacked 3% hydrogen peroxide treated crop however did not found attacking colchicine treated crops (Fig. 5 A). Aphids attacked on cowpea grown from seeds treated with 0.005% colchicine in distillated water occurred on day 24 after planting. Cowpea crop grown from seeds treated with 0.01% colchicine in 3% hydrogen peroxide was also attacked by aphids however on day 32 after planting or eight days later (Fig. 5 B).

It wasn't any bean leaf beetle (*Phaedonia inclusa*) symptoms found in cowpea crop grown from seeds treated with colchicine in 3% hydrogen peroxide up to 25 days after planting however symptoms of bean leaf beetle have appeared on cowpea crop treated with colchicine in distillate water 7 days after planting. Those results, although still require further evidence can be considered as promising preliminary result for developing crop resistant to soil worm and aphid through inducing mutagenesis using colchicine or other mutagens in hydrogen peroxide. Ranney and Jones (2008) stated that the increase in number of chromosomes could possibly increase the production of secondary metabolites and chemicals for plant self-deffence that will ultimately improve plant resistance to plat pests and diseases.

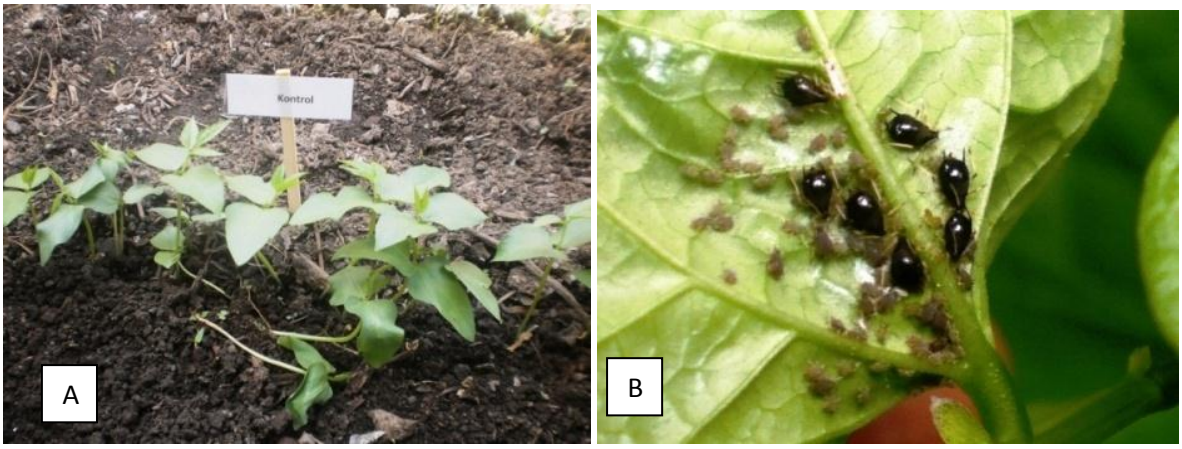

Fig. 5. Soil worm attacked control cowpea (A). Aphid attack on cowpea treated with colchicine 0,01% in H_2O_2 (B)

Furthermore, we found that seed treatment with colchicine 0.01% in 3% hydrogen peroxide on cowpea caused color pod change from green to purple (Fig. 6). Odekola and Oluleye (2007) has conducted a research inducing mutagenesis using gamma ray on cowpea resulted in mutant cowpea with dark green, twisted and hairy pod. We consider that pod color changing due to colchicine treatment of this research having high academic value and might be economic value as well. Purple pod of cowpea may contain more antioxidant and for sure will increase biodiversity.

Fig. 6. Pod color change to purple

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