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Abstract Drought stress causes yield losses of plant production in a tropical area like Indonesia. In vitro selection was a method to the identified somaclonal variation of dwarf Napier grass by using callus induction. The non ionic water soluble polymer polyethylene glycol (PEG) of molecular weight was used as osmoticum to simulate water stress for in vitro selection. This study aimed to use somaclonal variation to select drought tolerant plants of dwarf Napier Grass. Multiple shoot clump (MSC) of sterilized explant were cultured on Murashige and Skoog (MS) medium containing mgL dichlorophenoxyacetic acids (D ml l coconut milk) callus growth medium (CGM) with g l sucrose and g l phytagel for callus induction. The calli were grown on CGM with five concentration of PEG for selected medium µM µM µM and µM for weeks. There were significant differences between fresh and dry weights of callus in different concentrations of PEG. Callus regeneration of dwarf Napier Grass occurred at the concentration of and µMof PEG and all regenerated MSC well rooted on all concentration PEG. In order regenerated shoot occurred at the concentration more than µM PEG solutions.

Keywords *Callus Drought Tolerant In Vitro PEG Napier grass*

Introduction

Drought is a crucial problem in Indonesia ha dry land it is covering of marginal land and dry land in Sulawesi island. The negative impact of drought stress has been recognized to pose a growing threat to sustainable agriculture particularly under the global climate change. Water stress is a major factor of environmental stresses such as drought salinity and low temperature. Whereas – of the agricultural land around the world suffers from drought [ ]. To anticipate widely of the dry land regarding of climate change and reducing agriculture land for residential and manufacture sector construct new cultivar crop adapted to climate change is one of the important strategies for adapting agriculture to climate change. Breeding program by using biotechnology mostly needed for environmental engineering. Increase in drought periods may require the development of drought tolerant crops [ ].

Breeding for water stress tolerance by traditional methods is a time consuming and inefficient procedure. Tissue culture as a possible technology for obtaining the desired characteristics of variants can lead the variation to the expected outcomes while the probability accomplish an in vitro selection depends on the accessibility of an effective regeneration system associated with an efficient selective agent [and]. The most of popular forages in Indonesia is dwarf Napier grass its grown widely environment and climate. Dwarf Napier grass contains several positive characters which are beneficial for animal feeding i.e. they can be grown for years having high yield highly nutritious selectively chosen as feed by animals and can be grown in a wide variety of soil types. However there are several negative properties which prevent this grass to be developed as planned [ ].

Polyethylene glycol PEG is one of the most popular approaches is to use high molecular weight osmotic substances PEG used for selection callus and plantlet were in vitro drought stress induction PEG is a non penetrable and nontoxic osmotic substance which is used to lower the water potential of the culture medium and it has been used to simulate drought stress in cultured plant tissues [ ] Successful in vitro selection for drought tolerance using polyethylene glycol PEG sorbitol mannitol and agar as selection agents has been also applied to several crops such as rice sugarcane sun flower tomato and *Tagetes minuta* [and]

Selection drought tolerance by using tissue culture technique has been widely used for the breeding purpose [ ] The lack research on drought tolerance of Napier grass by using PEG in order there are several genera of grass were reported such as sugarcane sorghum and wheat [ ]

Method

Multiple shoot clump MSC from the clone of *Pennisetum purpureum* cv Mott were used to induce embryogenic callus Explant was sterilized in ethanol solution for minutes and burn on Bunsen for times [ ] To initiate embryogenic callus MSC cultured on solid MS medium Murashige and Skoog supplemented with mg l NAA α naphthaleneacetic acid mg l D dichlorophenoxyacetic acid mg l BAP benzilaminopurine v v coconut milk g sucrose and w v gelatin The inhibitory effects of polyethylene glycol PEG at µM µM µM and µM concentrations in MS medium on initiation callus were evaluated in a completely randomized design experiment Each experimental unit consisted of three explants cultured in one culture vial ml containing ml of MS medium Five replicates were prepared for each of PEG concentration This study used liquid PEG supplemented MS medium to initiate callus as agar would not solidify in the presence of a high concentration of PEG Sterilization of the media used standard heat sterilization methods at °C kg cm using an autoclave The EC were transferred to fresh medium every weeks

Results and Discussion

Callus induction was observed following weeks of culture The percentage of callus compact and viable showed the calli was growth Napier grass explants exhibited a significant interaction with PEG stress levels In control PEG almost all explants initiated callus With the increasing level of PEG the mean percentage of callus induction reduced significantly The increasing level of PEG from to µM µM µM and have the lowest effect of differentiation of callus rooting and shooting These results indicating their relative tolerance for drought stress whereas the highest effect of PEG on medium selection In vitro response of Napier grass callus to PEG stress was shown in Table There are three responses of explant from MSC after transferred to treatments medium by using PEG

Table Percentage Organogenesis of Dwarf Napier grass on Medium Selective Polyethylene glycol

Treatments (µM)	Induction callus	Shoots	Rooting
Kontrol	85.7	50	85,7
PEG K1	71.4	40	71,4
PEG K2	83.3	25	83,3
PEG K3	66.6	0	66.6
PEG K4	66.6	0	66.6

Description K Without PEG Control K µM PEG K µM PEG K µM PEG K µM PEG K µM PEG The observation was started since explant planted on callus initiation medium until organogenesis on regeneration medium age days Table Showed induction percentage on callus induction medium containing to µM µM µM and µM PEG was and respectively Generally the average of callus induction decreased under higher PEG treatments except in treatments µM the percentage callus induction higher than µM PEG The decrease in callus induction is a typical response of explants of crop genotypes when subjected to PEG stress Matheka et al Biswas et al Abdel Ghany et al To maintain osmotic balance to assist initiation of callus cells under severe stress conditions or might be due to either water shortage which led to profuse mutation in cellular metabolism including protein functioning and alteration in the number of proteins

The results of induction callus showed that somaclonal variation from embryonic callus might tolerance or resistance to environmental stress were modified using PEG Callus is the initial step in the cellular plant to initiate somatic embryogenic If embryonic callus not resistance to drought condition and water stress the callus will necrosis on PEG medium at least to day after callus induction In order the percentage of callus for shoot formation more decrease than the percentage of callus induction Table showed on medium treatments for µM and µM PEG not formation shoot but on medium µM PEG was respectively Generally the average of shoot formation is under on treatments medium

Next step after callus induction for in vitro selection was callus rooting Early and rapid elongation of roots is an important indication of drought tolerance Continued elongation of root under the situation of drought conditions was the remarkable character of resistant cultivars Until µM of PEG equivalent to g l concentration of PEG embryogenic explants ability to rooting for stress conditions This may be due to their initial ability to fight with water stress condition and stay green Initial rooting appears in weeks after callus induction Table Showed that all treatments of PEG concentrations rooting despite highest concentration of PEG decreased the number of roots Highest root percentage of treatments were recorded by drought resistant whereas and when tested at and µM PEG solution respectively The lowest percentage of root was similarly on medium with concentrations and µM of PEG

Studied showed that PEG solution not the inhibitor for rooting formation of Napier grass callus in order on shooting formation increasing concentration of PEG more than µM could not shoot formation Studies indicated that root is more affected by drought condition than shoot Balanced growth was observed in drought resistant of callus Napier grass on same treatments of PEG solution Drastic reduction in shoot growth was observed with increasing PEG concentration which was considerably lower in the percentage of the shoot

From these studied we resulted that without gamma irradiation induction of callus on PEG solution for assessing drought tolerant successfully in Napier grass under in vitro selection Tissue culture increased somaclonal variation therefore new clonal will appear under stress condition for genetic variation [ ] Selection on cell phase more effective for one s factors selection So the targeting of agent selection on in vitro selection such as drought tolerance salinity and disease under a controlled environment Therefore somaclonal variation generated by using in vitro selection allow mutation on cellular phase thus new variants that are genetically tolerant to the abiotic stress like water stress are generated in the initial phase of plant or cell formation [ ]

Conclusion

The average percentages of callus formation were on the selective medium of PEG solution in order concentration more than µM PEG decreasing percentage of rooting and shooting of callus dwarf Napier grass *Pennisetum purpureum cv Mott*

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