In Vitro Selection of Salinity Tolerance Callus of Dwarf Napier Grass (*Pennisetum purpureum* cv. *Mott*)

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**Abstract:** Tissue culture for forage is a multiplication by improving the quality genetic of forage and adaptation to abiotic stress condition such as salinity stress. In this idea, different levels of salinity tolerance were generated. Multiple shoots as initial explants were isolated from aseptically shoot-tillers in the field, and cultured in vitro. Explants were induced into induction calli medium. Explants were transferred to Murashige Skoog (MS) medium, added with 5% coconut water and 2 mgL⁻¹ 2,4-D (*dichlorophenoxyacetic acid*). The planted explants were incubated for 21 days. Calli were induced in the proliferation medium with 50 μM CuSO₄ and growth regulators i.e 0.1 mg L⁻¹ 2,4-D, and 2 mg L⁻¹ BAP for 40 days. Regeneration medium with addition, and growth regulator 0.1 mg L⁻¹ NAA and 2 mg L⁻¹ BAP for 30 days. In the medium of proliferation and regeneration were added different salinity (NaCl) level. The treatments were 42.5 mM NaCl, 85 mM NaCl, 170 mM NaCl. The percentage of green-spot production, root formation, shoot, complete plantlet, morphologically of calli were recorded. The results indicated that morphologically and physiologically of calli added NaCl concentration (salinity) influenced growth on each treatment of dwarf napier grass (*Pennisetum purpureum* cv. *Mott*)

**Keywords:** Dwarf napiergrass, in vitro culture, NaCl, salinity tolerance

1. **Introduction**

   Indonesia is estimated salinity of 440,300 ha with salinity criteria of 304,000 ha and a salinity of 140,300 ha [1]. Most of lands that have high salinity levels can not be used as productive land for planting and only adversely affect plant growth. Among the variety of environmental stresses, salinity is one of the most common stresses [2] which can be utilized. One of the strategies of saline land is tolerant varieties with the selection of plants that have environmental adaptation and tolerance to live in the saline soil. Tissue culture is one of vegetative propagation of plants, by isolating parts of plants and growing in aseptic-rich, nutrient-rich medium and growth regulators [3] by totipotency cell principle [4]. Tissue culture on forage into breeding and genetic improvement by genetic quality of forage in vitro. Increasing the quality of forage particularly grass can be increased by increasing crop adaptation to abiotic stress conditions such as drought and salinity [5]. The mechanism of plant tolerance to salinity requires to be understood from the morphological, physiological, and biochemical aspects of the plant as a strategic step in developing a tolerant cultivar of saline soil as well.
Based on the results of the study [6], dwarf napier grass callus grown on medium with gamma irradiation treatment with NaCl concentration of 0.5-1.0% NaCl successfully established plantlet with tissue culture technique. Furthermore [7], tolerance of dwarf napier grass calli (Pennisetum purpureum cv. Mott) at various PEG concentrations up to 1000-4000 ppm. The abiotic environment research by tissue culture technique on dwarf napier grass (Pennisetum purpureum cv. Mott) was to test the presence of callus that can survive on salinity medium.

The aim of this study was to examine the presence of dwarf napier grass (Pennisetum purpureum cv. Mott) calli which can grow in different NaCl concentrations and is expected to be dwarf napier grass (Pennisetum purpureum cv. Mott) resistance to abiotic stress salinity. By this research, in vitro abiotic environmental engineering of dwarf napier grass (Pennisetum purpureum cv. Mott) will produce more saline-tolerant saline to be consumed as forage for ruminants although it was developed in the salinity land.

2. Material and Methods

The research was conducted from August 2017 to January 2018, in Laboratory of Bio Science and Biotechnology of Plant Reproduction Teaching Industry of Hasanuddin University, Makassar, Indonesia. Research procedures were sterilization, initiation callus medium, sterilization explant, and initiation of callus. Explant grown on induction medium callus and subcultured on proliferation medium. This step, the explant that has been planted and stored in the incubation room for 40 days, observed the callus changes and subcultured on the regeneration medium. The treatment for regeneration medium were: P0: Media MS + 5% coconut water + 0.1 mg L^{-1} NAA + 2 mg L^{-1} BAP + without NaCl concentration (control) P1: MS medium + 5% coconut water + 0.1 mg L^{-1} NAA + 2 mg L^{-1} BAP + 42.5 mM NaCl ; P2: Media MS + 5% coconut water + 0.1 mg L^{-1} NAA + 2 mg L^{-1} BAP + 85 mM NaCl ; P3: Media MS + 5% coconut water + 0.1 mg L^{-1} NAA + 2 mg L^{-1} BAP + 170 mM NaCl. This stage of planted explants was stored in the room incubation for 30 days and observed the callus changes that persisted at different NaCl concentrations. Parameters observed in this research were; morphologically and physiologically of calli, callus types and percentage of rooted callus.

3. Results

3.1. Callus Morphology (Pennisetum purpureum cv. Mott) on Salinity

Callus morphological changes can also show the growth of dwarf napier grass explants (Pennisetum purpureum cv. Mott) into callus that can be seen from the shape, color and response of callus. More specifically, callus morphology on treatments respectively is presented in Table 1.

| No | Treatments | Callus shape on Medium Initiation | Callus Respon on Medium Initiation | Callus Color on Medium Initiation |
|----|-------------|---------------------------------|-----------------------------------|----------------------------------|
| P01 | Root Formation | +++ | Brown |
| P02 | Root Formation | +++ | Brown |
| P03 | Root Formation | +++ | Brown |
| P04 | Friable | ++ | Brown |
| P05 | Compact | +++ | Brown |
| P11 | Compact | +++ | Brown |
Description: The observations were performed for 21 days on callus initiation medium and 30 days on Proliferation Medium. (+++) Callus grows on part of surface explant. (+++) Callus grows on the surface explant.

3.2. Callus Types of Dwarf Napier Grass  (*Pennisetum purpureum* cv. *Mott*)

Based on the treatment observations, the average form of callus organogenesis is derived from the formation of roots. As for the form of dwarf napier grass callus (*Pennisetum purpureum* cv. *Mott*) is presented in Figure 1.

![Callus Types](image)

**Figure 1.** Callus Types (A). Friable callus. (B). Compact callus (C). Root formation callus (D). Browning callus. (E). Contaminated callus.
3.3. Growing Percentage of Dwarf Napier Grass Callus (Pennisetum purpureum cv. Mott)

Callus growth can be measured by the percentage of callogenetic callus growth. The average percentage of growth of dwarf napier grass callus (Pennisetum purpureum cv. Mott) can be seen during being on callus initiation medium until organogenesis on regeneration medium which is presented in Table 2;

Table 2. Growing Percentage average of Dwarf Napier Grass Callus (Pennisetum purpureum cv. Mott)

| Treatments | Percent of Green spot | Percent of rooted callus | Percent of sprouted callus | Percent of plantlet callus |
|------------|-----------------------|--------------------------|---------------------------|---------------------------|
| P0         | 100                   | 100                      | 60                        | 60                        |
| P1         | 100                   | 100                      | 60                        | 60                        |
| P2         | 100                   | 100                      | 40                        | 40                        |
| P3         | 100                   | 100                      | 0                         | 0                         |

Description: P0 (Without NaCl addition); P1 (42.5 mM NaCl); P2 (85 mM NaCl); P3 (170 mM NaCl). The observation was started since explant planted on callus initiation medium until organogenesis on regeneration medium age 30 days

4. Discussion

4.1. Morphology of Dwarf Napier Grass Callus (Pennisetum purpureum cv. Mott)

The effect of salinity treatment which is given on planting medium is presented in morphology of callus. Measurement of morphological and physiological characters is one approach to study how the effect of salinity stress on growth and production. This information can be applied in the selection of salinity-tolerant plants causing the lack of water [8]. Morphological changes in callus can show the growth of dwarf napier grass explants into callus indicated by he shape, color and response of callus as well. The growth of explant into callus consists of several stages. Starting from the swelling of callus and the appearance of white cells that are suspected to be a response of formation callus.

Based on Table. 1, there are several shapes, colors, and responses to the treatment given. Every treatment has the yellowish white color on the initiation medium. However, once transferred to proliferation medium (6-7 days), the color of the calli are browning after transferred in regeneration medium. Browning can occur in dwarf napier grass callus because dwarf napier grass contains phenolic compounds when oxidized with O₂, formed the quinon compound [9]. When the callus cells are injured, new cells will cover the wound and phenol compounds will accumulate in these cells begin to harden. Oxidation of phenol will produce quinon organic compounds because of browning [10].

Callus opening occurs at subculture time, so that the accumulated phenol compounds caused callus brown. Some protoplas flows out so as to start form callus [11]. Based on this study, the addition of NaCl in the medium resulted in brownish calli. P3 treatment experienced callus color changes deeply. Callus color increasingly dark brown to blackish and finally to be rotten. Oxidation of phenol that turns into a highly toxic quinone causes browning of the medium and explant death in line with escalating NaCl concentration [12].
Based on Table 1, each treatment has same callus form namely compact, friable, and root formation. Callus form at each treatment is different. They have different character, different growth rate and different ability of the tissue to absorb nutrients and growth regulator substances in the culture medium.

Several studies on the effects of salinity stress on plants have been used widely. Shoot growth in lamtoro seedlings (*Leucaena leucocephala*) decreased by 60% with salinity addition to a medium about 100 mM NaCl [13]. Salinity decrease the concentration of Fe$^{2+}$ ions in leaves and roots [14]. The decrease is due to the reduced absorption of Fe in high salinity conditions. Based on Table 2 calli color have a considerable similarity, but have different responses. Calli response rate will be higher on the callus in the form of root formation. Based on observations on the treatment of P0, P1, P2, and P3. The average form of organogenesis callus forming plantlet is callus derived from root formation.

4.2. Callus Types of Dwarf Napier Grass (*Pennisetum purpureum* cv. Mott)

Based on Figure 1 each treatment has different types of callus A). Friable callus. B). Compact callus (C). Root formation callus. Callus growth is influenced by 2,4-D growth regulator. 2,4-D is effective for stimulating callus formation because of strong activity to stimulate cell differentiation, organogenesis and callus growth. Root formation callus results is more better growth rather than other callus forms. About 60% for growth of shoots and plantlets [15]. Callus cultures have varying morphogenetical potency. Callus from some plant species or from some explants, often fails to regenerate shoots or form only roots [16]. The different types of callus are also based on the difference in the success of embryogenesis.

Exceptions to image D). Callus browning. E). Callus contaminated. Figure 1 (D) shows there are callus that have browning. Browning on old callus explants and medium culture around the callus became a problem in the initiation and proliferation phases [17]. (E) Shows contamination of the callus. Contamination is one of the factors in developing disorders, explant can die before growing into a plantlet. The types of contaminants found are fungi and bacteria. These types of contaminants can be identified by their physical appearances. Usually, the surface of the bacterial colony is slippery, whereas rough fungi is fiberous. The most common contaminants found in cultures are bacteria. Contamination of callus on induction medium of callus of Dwarf napier Grass (*Pennisetum purpureum* cv. Mott) is caused by sterilization or contaminated explants.

4.3. Percentage Growing Dwarf napier grass Grass Callus (*Pennisetum purpureum* cv. Mott)

Based on the data presented in Table 2 indicated that the average of the treatments have root formation callus and green spots. The callus on each treatment organogenesis by showing the growth of roots and green spots until 100%. Callus growth was based on the addition of 2,4-D growth regulator on the induction medium of dwarf napier grass callus (*Pennisetum purpureum* cv. Mott). The use of 2,4-D auxin can stimulate callous growth [18]. The percentage of explant form callus is also related to the time of callus formation, the faster the callus time formed on explant, the greater the percentage of callus that formed [19].

The treatments indicated that the percentage of shoots and plantlets. The average of each organogenesis-shaped treatment shoots will form a plantlet as well. The highest percentages formed shoots and plantlets on treatment P0 and P1. The growth of shoots and plantlets decreased with increasing salt concentration on P2 and P3 treatments.
5. Conclusion

The average percentage of shoots and plantlet growth was highest P1 in treatment (Addition 42.5 mM NaCl) from callus formation of root formation. Morphologically and physiologically, giving NaCl concentration gives a salinity effect that affects growth on treatment of dwarf napier grass (*Pennisetum purpureum cv. Mott*) respectively.

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7. References

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