IOPICOBIO_Callus response of hybrid

by Kelik Sukma

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Callus response of hybrid and Madura local corn to salt stress

K P W Sukma[1], B S Daryono[2]*, Purnomo[2] and I Suprapti[3]

[1]Study Program on Agrotechnology, Faculty of Agriculture, Universitas Islam Madura Jl. Ponpes Miftahul Ulum Bettet Pamekasan, East Java, Indonesia,
[2]Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3]Study Program on Agribussines, Faculty of Agriculture, Universitas Trunojoyo Madura, Indonesia.

*Corresponding author: bs_daryono@mail.ugm.ac.id

Abstract. Salinity (salty and acidity) is one of important abiotic stresses, which can decrease corn production. In Indonesia, corn is one of important cereal crop beside rice. Madura is an island that known as widest area of maize landfill in East Java but has lowest productivity. Madura local corns (var. Manding, Duko and Elos) and hybrid corn (var. Sukmaraga) known as salt tolerance. This research objectives was to compare response of those callus to salt stress. Callus responses to salt stress, can indicate to their resistance to salinity. The young corn stalks were induced to be callus by MS+2,4D 4mg/L, then cultivated to media MS+NaCl (0, 100, 200 mM) for 30 days. The parameters were morphology (colour and texture), survived and proline content. Proline content was measured by nynhidrin methods. The results showed that salt stress change callus colour (from white to creamy white and brown) and texture (from friable to compact and intermediate). Duko and Elos survived more than 90%, but Manding and Sukmaraga survived less than 15%. Salt treatment increased proline content of all callus. Madura local corn had better tolerance to salt stress than hybrid.
Keywords: Callus, salt, hybrid corn, local corn, proline

1. Introduction

Corn (*Zea mays* L.) is one of the most important cereal crop besides rice and wheat. Corns can cultivated on a wide spectrum of soil and climatic conditions. In Indonesia, corn is the second most important cereal crop after rice. Madura island known as widest area of maize landfill in East Java, Indonesia. There were 16 varieties of corn in Madura, such as ; Tambin-1, Tambin-2, Delima, Cetek, Dheber, Parsong, Krajekan, Elos, Talango, Kangean, Guluk-guluk, Dlubeng, Raddin, Manding, Geltik and Duko [1]. They have superiority on short time harvest (65-75 days), but its inadequacy on productivity (1,4 tons/hectare). Low productivity was caused by low rainfall, marginal land and unselected seed [2].

Marginal land in Madura Island are drought and saline land [3]. Ssalinity is one of important abiotic stresses that can interfere agricultural sustainability. Land become saline naturally occurs in coastal swamps, divided into two categories, primary and secondary. The primary occurs if salt has been a part of soil composition from the beginning of soil forming. The secondary occurs when low salinity soil becomes higher due to lack of water and poor drainage [4]. Salt stress is caused by high level of land salinity. It is affects physiological and biochemical pathway in the plants by complex

mechanism [5, 6]. Salinity is one of the main limiting factor that can decrease growth and productivity of many plant species [7] include corn [8].

Those are the reasons that research the tolerance of many varieties of corns to salinity stress important to do. There were many varieties known as salinity tolerance such as research of [9, 10-13]. Madura corns that were known have tolerance to salinity are Manding [14], Elos and Duko [15]. The corns should be improve genetic quality. In vitro selection is one of the effective technique to screen salinity or salt tolerance corn varieties [16]. The using of in-vitro media can help to learn the salt tolerance mechanism physiologically and biochemically. The selection result can be developed to have more seed that have stress tolerance. Callus respons can become basic information to know the tolerance level of corns to salinity. So, this research objective was to compare the response of the local madura and hybrid corns callus at saline medium.

2. Material and methods

2.1. Plant material and research design

Corns for the research used hybrid and Madura local corns. Madura local corns that were used from the research of [14, 15] such as var. Duko, Elos and Manding. Hybrid maize was Sukmaraga as comparator from Research Center of Cerealia Maros, South Sulawesi, Indonesia [17]. Seeds with similiar size and weight were selected to obtain homogenous germination rate. Seeds were sterilized in 2% (v/v) NaOCl for ten to fifteen minutes and washed by distilled water then were placed on MS (Murashige Skoog) medium [18].

The experiment was conducted by using completely randomized design with 4 replications. Significant differences among treatments were determined by Duncan's Multiple Range Test at α 5% level.

2.2.Callus induction

The mature embryos (72 hours of incubation) were removed from the seeds, then it was longitudinally sliced into halves and inoculated on MS [18] + 2,4-D 4mg/L. The induced callus (30 mg) were then put on MS + 2, 4-D 4 mg/L + NaCl (0, 100, and 200 mM). Then it were incubated in dark room at 25±2°C for 30 days.

Number of alives and morphology of callus were observed after 30 days incubation. Callus weight measured before and after incubation. Proline content on callus conducted by [19].

3. Results and discussions

3.1. Callus character

Table 1 shows that the callus of all varieties could growth on MS media (NaCl 0mM). They had good colour (could not had browning) and texture, means callus had living activity. Morphology changed in salt treatment. In 100mM NaCl Callus colour of Manding, Elos and Sukmaraga had little browning or changed to yellow or creamy white. In 200mM NaCl, Manding and Sukmaraga callus had browning. The texture became intermediate and compact in 200mM salt treatment.

The difference of morphology showed that NaCl stress in a certain period of time decrease physiological function [20] or can make cell become death [21]. The accumulation of excessive Na^+ and Cl^- ions cause inability to regulate the concentration then consist plasmolysis. The plasmolysis can see from compact callus caused by the plasms leaved out to cells [22].

Callus Browning due to the presence of phenol compounds [23], which is toxic to cells. Tissue browning occurs due to activity of polyphenol oxidase and tyrosinase (one of copper-containing oxidase enzymes group). The presence of salinity stress interfere the secondary metabolite pathway and increase phenol compounds as an adaptation mechanism to salinity stress [24, 25].

3.2. Callus lives or survive and growth

Figure 1 shows that increasing of salt stress reduced callus surviving. Callus of Manding and Duko 100% lives on 100 mM salt media, but Elos and Sukmaraga decreased although not significantly differences with control. Callus of Duko and Elos were the highest percentage of callus live in 200mM. The result less difference with [14] that the callus (Manding and Bluto) could survive 100% on 7500 ppm NaCl (±128,3mM). The percentage of living callus conform with the morphology on salt stress. According to Sajid and Aftab [26], that change of callus morphology (browning to black) may be linked to cell death.

Table 1. Callus morphology of local Madura and hybrid maize on difference salinity level

Parameter	Varieties	NaCl Treatment		
		0 mM	100 mM	200 mM
Browning	Manding	-	+	++
	Duko	-	-	-
	Elos	-	+	-
	Sukmaraga	-	+	-
Texture	Manding	F	F	C
	Duko	F	F	I
	Elos	F	F	I
	Sukmaraga	F	C	C

Notes : F = Friable, C = Compact, I = Intermediate of Friable and Compact

Salt stress was not just affected on morphology and survival of cells, but also affected on cell growth. Figure 2 show that salt stress decrease callus growth all of varieties. Growth decreasing has also been reported by previous research in the other species such as, potato [26, 27], *Chrysanthemum morifolium* [28] and *Jatropha curcas* [29]. Growth decreasing due to metabolism decreased. It's one of the strategies of higher plants to slow down their growth and metabolism [30].

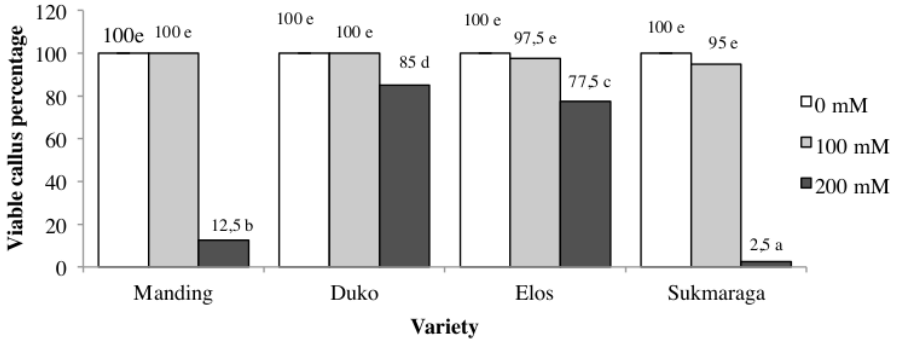

Figure 1. Percentage of corn live callus on salt stress

The survival of callus on salt stress concern with tolerant or avoidance response. All mechanism concern with ion balancing in the plant cell or organ. Plant respons salt stress by produce some osmolites [31] such as peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and others [32-35] or prolin [15] and betain [36] to scavenge reactive oxygen species (ROS) leading to plant cell from damage [37, 38] . This research also showed that NaCl treatment increase prolin content (figure 3).

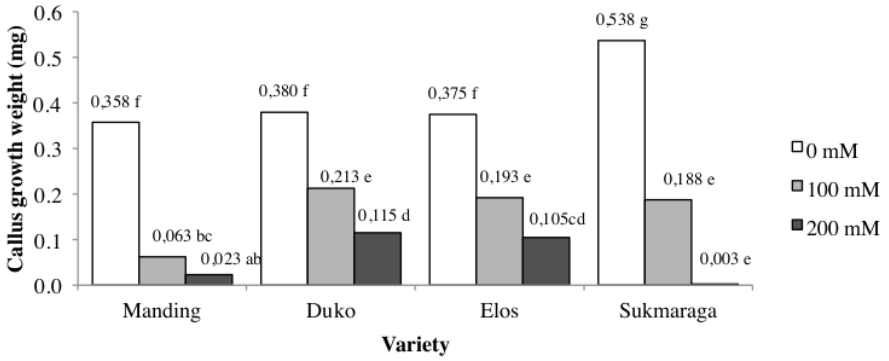

Figure 2. Corn callus growth on salt stress

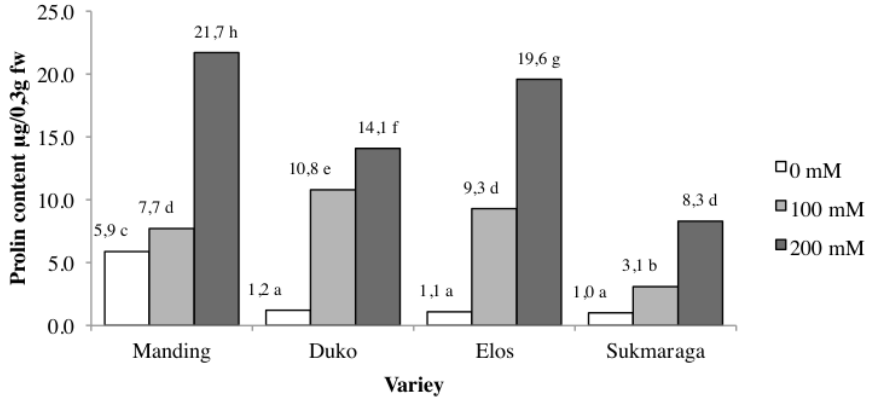

Figure 3. Prolin content of callus on salt stress

4. Conclusion

Salt stress change callus colour (from white to creamy white and brown) and texture (from friable to compact and intermediate). Salt stress increased proline content of all corn callus. Madura local corn had better tolerance to salt stress than hybrid.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5