Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(1): 152-156. **OPEN ACCESS**

Molecular genetic markers associated with salt stress in local and hybrid corn

Kelik Perdana Windra Sukma[1], Budi Setiadi Daryono[2*], Purnomo[2], and Isdiana Suprapti[3]

[1] Faculty of Agriculture, Islam Madura University, **Indonesia**
[2] Faculty of Biology, Gadjah Mada University, **Indonesia**
[3] Faculty of Agriculture, Trunojoyo Madura University, **Indonesia**

***Correspondence: bs_daryono@mail.ugm.ac.id** **Received 08-11-2019, Revised: 15-01-2020, Accepted: 21-01-2020 e-Published: 23-01-2020**

Corn is the second most important cereal crop after rice. Salinity, drought, alkalinity, temperature, and mineral toxicity is one of major limiting factors on plant production include corn. This research objective was to study molecular marker RAPD associated with salt tolerance on local Madura (Var. Elos and var. Duko) and hybrid corn (var. Anoman and var. Sukmaraga). Those corns were planted by wick system hydroponic for 3 weeks. Media used as MS + NaCl (0, 100, and 200mM). DNA was isolated by nucleon phytopure protocol then amplified using PCR. The amplified fragments profile was defined by the presence (1) and absence (0) at particular positions on the gel. Cluster analysis and dendrogram had been done by Unweighted Pair-Group Method Arithmetic (UPGMA) used Multi-Variate Statistical Package (MVSP). The results were show that the corns which had tolerance to salt stress were in the same forks or in one group. Its found 120 scorable amplified DNA fragments ranging in size from 139 to 1772 base pairs. Seven positive primers were found to be associated with salt stress, while six negative primers found.

Keywords: corn, RAPD, salt, primers, fragments

INTRODUCTION

Corn (*Zea mays* L.) is one of the most important cereal crop beside wheat and rice, which is grown under a wide spectrum of soil and climatic conditions (Chinnusamy *et al.* 2005). In Indonesia, corn is the second most important cereal crop after rice. Madura island is known as the widest area of corn landfill in East Java, Indonesia. Sixteen Madura local corns have identified. Four cultivars have higher production potential than others, namely Tambin-1, Delima, Tambin-2, and Raddin. Krajekan have the shortest harvest time Amzeri (2010). Manding, Duko and Elos varieties have the potential as salinity resistant (Sholihah and Saputro, 2016 ; Sukma *et al.* 2018).

Salinity is one of major limiting factor on plant production (Flower, 2004) beside drought alkalinity, temperature, and mineral toxicity (Munns and Tester, 2008). Salinity influences plants by several mechanisms such as inhibit water uptake (Sukma *et al.* 2018) due to cell osmotic mechanism, ion toxicity due Na^+ and Cl^- (Chinnusamy et al., 2005) and nutritional imbalance (Abbasi *et al.* 2016).

Eforts to raise corn production have been made by improving the environment and genetic quality. Genetic quality can be improved by breeding them to become more tolerance to abiotic stress. But it have many challenges because the mechanism and procedure of plant to face stress is controlled by many genes (Flower *et*

al. 2004).

Molecular marker usage is one of the tools to detect genetic variation among plant species, populations and varieties and to find marker related to special traits (Mahgoub *et al.* 2016). Molecular markers such as RAPD (randomly amplified polymorphic DNA), ISSR (inter simple sequence repeat), SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) and isozymes can be used to assist selection of QTLs (quatitative trai loci) associated with traits (Xue *et al.* 2009). This research would study molecular marker RAPD associated with salt tolerance on local Madura and hybrid corn.

MATERIALS AND METHODS

Corn ware took from the research of Sukma *et al.* (2018), var. Elos (E), Duko (D). The hybrid corns were salt tolerance (Sukmaraga/S) and salt-sensitive (Anoman-1/A) from Research Center of Cerealia Maros, South Sulawesi, Indonesia. Seeds with similar size and weight were selected to get the same germination rate. Seeds were surface sterilized in 2% (v/v) NaOCl for 10 min and washed by distilled water. Thirty seeds were placed on filter papers that contained NaCl solution (0, 100, 200, mM) and located in 15 cm diameter steril petridishes. The germinated seed then cultivated by wick system hydroponic. The media used Murashige&Skoog (MS) media (1962)

DNA isolation and PCR amplification

Genomic DNA was extracted from corn leaves three weeks days old salt treated using Nucleon phytopure DNA extraction procedure. Primers used were seven (Table 1) from different sets which was reported had unique band by Balkrishna and Shankarrao (2013).

Table 1. Primers used for RAPD analysis

S/N	Primer	Primer Sequence (5'-3')	Annealing Temp.(ºC)
1	A11	CCT GTT AGC C	38.1
2	C20	ACT TCG CCA C	38.1
3	OPA10	GTG ATC GCA G	32.6
4	OPA13	CAG CAC CCA C	34.5
5	OPK20	GTG TCG CGA G	34.5
6	OPX11	GGA GCC TCA G	32.6
7	OPI01	ACC TGG ACA C	36.7

Premix PCR was made by mixed 10.5 µl ddH_2O steril, 12.5 µl Bioline 2x My Taq HS hotstart, 20 ng Primer RAPD, 50 ng *DNA template* A 25 µl. Amplifications were carried out using Bio RAD T100 thermal cycler, programmed for 40 cycles as follows: pre-denaturation at 95°C for 3 min, further denaturation at 95°C for 30 min, annealing for 1 min, elongation at 72°C for 1 min and post elongation for 8 min at 72°C. Temperature used for annealing depend on primers (Table 1).

The PCR products were separated at 100V for 4h on 2% agarose gel prepared in 1x Tris-borate-EDTA (TBE) buffer. Gel was photographed using Gel-Documentation system (ALPHAIMAGER TM 2200). Each amplified band profile was defined by presence (1) and absence (0) at particular positions on the gel. Cluster analysis and dendrogram had been done by Unweighted Pair-Group Method Arithmetic (UPGMA) used Multi Variate Statistical Package (MVSP).

RESULTS AND DISCUSSION

Cluster Analysis and Dendogram

Figure 1 showed that the corns which had tolerance to salt stress were in same forks or in one groups (S100-S200, E100-E200, D100-D200 and A100-A200). It means that the corns had similar genes which responsible to face salt stress.

Interesting result showed on that var. Anoman (sensitive to salt stress) was in the same group with local corn that had known as salt tolerance corn. It means that var. Anoman had more similar genes with local corn than var. Sukmaraga. It's guessed that similar genes did not relate to salt stress.

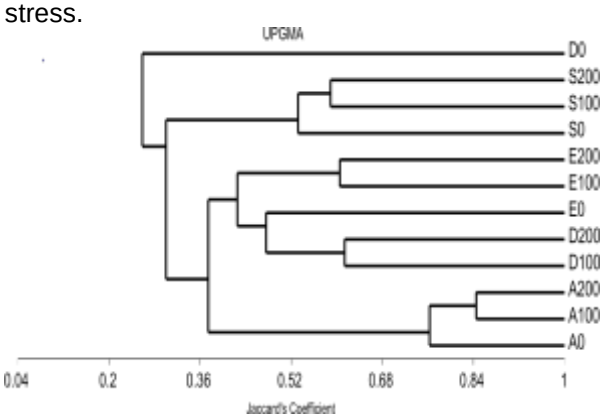

Figure 1. Dendrogram of Local and Hybrid Corn treated by salt stress.

Genes Associated With Salt Stress

There were 120 scorable amplified DNA fragments (Table 2) of 7 primers (Table 1.) ranging in size from 139 to 1772 base pairs. It's fewer than Balkrishna and Shankarrao (2013) that

reported 238 scorable DNA fragments from the same seven primers. On this research, OPA 13 was the most fragment and C20 was the fewest. 117 fragments (97,5%) were polymorphic. 3 primers showed monomorphism (A11, OPA10, OPX11) and 4 primers 100% polymorphism (C20, OPA13, OPK20, OPI01).

Table 2. Polymorphism percentage and range amplified bands of seven primer

Primer	Number of loci amplified	Polymorph-ism (%)	Range amplified
A11	16	93,8	190-1548
C20	14	100	381-1198
OPA10	15	93,3	181-1243
OPA13	22	100	140-1772
OPK20	17	100	254-1500
OPX11	18	93,3	173-1768
OPI01	18	100	449-1731

There were two group of fragments (band amplified), positive and negative. A positive induce fragments is a band generated in salt treated. Negative induce fragments is a band not generated on salt stress. The existence of fragments on salt treatment could be assumed that its associated with salt stress.

Table 3, show that there were 59 positive induced fragments that exist in salt stress treatment (100 and 200 mM NaCl). Mostly induce fragments were from var. Elos, Duko and Sukmaraga that are known as salt tolerance corn. Fragments of Var. Anoman existed in A11 primer (511 bp) and OPA10 primer (389 bp). 21 of 59 positive induced fragments were from intervariety comparison, which means that just existed in salt tolerance corn.

Positive induced fragment of corn on salt treatment was also reported by Balkrishna and Shakarrao (2013). They analyzed molecular genetic markers associated with salt tolerance in corn calli. Its were found 10 positive unique bands from six primers such as A11, C20, OPA 10, OPA 13, OPI01, OPK 20. Abdel-Tawab *et al.* (2001) found two positive and two negative molecular markers related to salt stress.

Table 4, show that there were 39 negative induced fragments that did not exist in salt stress treatment (100 and 200 mM NaCl), and were also from var. Elos, Duko and Sukmaraga that are known as salt tolerance corn. Although var. Anoman was known as salt sensitive variety but it less found negative induced fragment. It's suspected that var. Anoman had few genes associated with salt stress. Balkrishna and Shakarrao (2013) also reported two negatives markers (OPA 20 and OPX 11) associated with salt stress. Younis *et al.* (2007) found 10 negative markers related to salt stress on Sorghum.

Many plants have also been studied its molecular marker associated with salt stress used RAPD, such as alfafa (Yang *et al.* 2005), sorghum (Rao *et al.* 2007), barley (Abdel-Hamid, 2014), soybean (Khan *et al.* 2013 ; Mahgoub *et al.* 2016), sugarcane (Patade *et al.* 2005 ; Gadakh *et al.* 2017).

Table 3. Positive induced amplified bands of seven primers (intra-variety comparison)

Primer	Positive Induced amplified bands	Base pair (treatment)
A11	10	**250** (D200, E100, S100, S200), **292** (D100), **352** (A200), **511** (A100,A200), **602** (D100, D200, E100, E200), **701** (E100), **803** (E100, E200, S200), **901** (E100), **1011** (E200),**1115** (E100, E200), **1310** (E100, E200), **1400** (E100, E200), **1548** (E100, E200)*
C20	7	**414** (D100, D200)*,**444** (D100, D200),**501** (D100, D200)*,**539** (D100, D200),**589** (D100, D200), **871** (D100, D200),**1146** (D100, D200, S100, S200)
OPA10	6	**279** (D200)*, **389** (A100,A200),**877** (E200), **961** (D100, D200,S200), **1138** (D200, E200), **1243** (D200, E100, E200)
OPA13	8	**189** (D200)*, **317** (S100, S200)*, **450** (S200),**479** (E100,E200)*,**604** (S200), **633** (E100, E200), **740** (S100, S200), **802** (D200)*
OPK20	8	**374** (E200)*, **467** (E100, E200), **486** (E100, E200), **739** (D200), **812** (D100, D200)*, **844** (E200),**877** (D100,D200),**993** (E100,E200)*
OPX11	10	**332** (E200)*, **404** (E200)*,**649** (D100, D200),**777** (D100, D200),**904** (S200)*, **1025** (D200, E200), **1119** (D100, D200, E200), **1330** (D100, D200, E100, E200), **1399** (E200)*, **1533** (E200)
OPI01	10	**548** (E200)*, **600** (D100, D200), **683** (E200)*, **760** (E200), **893** (E100, E200)*, **974** (E100, E200)*, **1030** (E100, E200), **1100** (E100, E200)*, **1300** (E100, E200)*, **1731** (E200)*

* intervariety comparison

Table 4. Negative induced amplified bands of seven primers

Primer	Negative Induced amplified bands	Base pair (treatment)
A11	2	**451** (S100, S200)*, **602** (S100, S200)*
C20	4	**539** (S200)*, **579** (S200)*, **802** (S200)*, **982** (E100, E200)*, **1198** (E100, E200, S100, S200)
OPA10	6	**181** (D100, D200, E100, E200, S200), **217** (D100, D200, E100, E200, S200), **308** (A100, A200, D100, D200, E100, E200), 3b17 (S100, S200), **328** (E100, E200), **877** (S100, S200)*
OPA13	8	**866** (E100, E200)*, **740** (D100, D200), **690** (E100, E200)*, **551** (S100, S200)*, **479** (S200), **450** (E100, E200), **293** (E100, E200, S100, S200), **189** (E100, E200)
OPK20	9	**1500** (S100)*, **1160** (S200)*,**877** (E100,E200, S100, S200)*, **844** (S100, S200), **812** (E200), **739** (S200), **618** (S100, S200), **486** (A100, A200), **467** (S100, S200)*
OPI01	3	**1167** (S100, S200), **936** (D100, D200), **449** (S100, S200)*

* intervariety comparison

CONCLUSION

The using of the seven primers RAPD could describe molecular marker associated with salt stress on hybrid and Madura local corns. Its found 120 scorable amplified DNA fragments ranging in size from 139 to 1772 base pairs. Seven positive primers were found to be associated with salt stress, while six negative primers found. The corns which had tolerance to salt stress were in the same forks or in one group (by cluster analysis). Similarity level of among varieties didn't indicate their tolerance to salt stress.

CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

ACKNOWLEGEMENT

Thanks for the Ministry of Research, Technology and Higher Education (Kemerinstekdikti) of Indonesia that have given research grant on year 2018-2019.

AUTHOR CONTRIBUTIONS

KPWS dan IS designed and performed the experiments and also wrote the manuscript. BSD as a correspondence author and P performed DNA isolation, PCR amplification and data analysis. All authors reviewed the manuscript and approved the final version.

REFERENCES

Abbasi H, Jamil M, Haq A, Ali S, Ahmad R, Malik Z, Parveen, 2016. Salt stress manifestation on plants, mechanism of salt tolerance and potassium role in alleviating it: a review. Zemdirbyste-Agriculture. 103(2): 229–238. doi 10.13080/z-a.2016.103.030

Abdel-Hamid AME, 2014. Physiological and molecular markers for salt tolerance in four barley cultivars. European Sci. J. 10 (3): 252-272.

Abdel-Tawab FM, Rashed MA, El-Domyati FM, Salem TZ, Azer SA, Khufaga AF, 2001. Marker assisted selection for salt tolerance in corn (*Zea mays* L.). Egypt J. Genet. and Cytol. 29:175-181.

Amzeri, A. 2010. Characterization and Collection of Germplasm to Prevent Genetic Erosion of Madura Local Maize. Final Repot of Doctoral Dissertation Grant. Unpublished

Balkrishna RA. and Shankarrao SS, 2013. In vitro screening and molecular genetic markers associated with salt tolerance in corn. African Journal of Biotechnology. 12(27) : 4251-4255. Doi. 10.5897/AJB2012.2992

Chinnusamy V, Jagendorf A, Zhu JK, 2005. Understanding and improving salt tolerance in plants. Crop Sci. 45 : 437–448. doi:10.2135/cropsci2005.0437

Flowers TJ, 2004. Improving crop salt tolerance. J Exp Bot. 396 (55): 307-319.

Gadakh SS, Patel DU, Singh D, 2017. Use of RAPD Markers to Characterize Salt and Drought Lines of Sugarcane Int. J. Adv. Res. Biol. Sci. 4(5): 50-57. DOI: 10.22192/ijarbs

Khan IA, Bibi S, Yasmin S, Khatri A, Seema N, 2013. Phenotypic and genotypic diversity investigations in sugarcane for drought tolerance and sucrose content. Pakistan Journal of Botany 45(2): 359–366.

Mahgoub HAM, Sofy AR, Abdel-Azeem EA, Abo-Zahra MS, 2016. Molecular Markers Associated with Salt-Tolerance of Different Soybean (*Glycine max* L.) Cultivars under Salt Stress. Int. J. Adv. Res. Biol. Sci. 3(8): 241-267

Munns, R and Tester M, 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology. 59 : 651-681

Murashige T, Skoog M, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant. 15:473-497

Patade VY, Suprasanna P, Bapat VA, Kulkarni UG, 2005. Selection for abiotic (salinity and drought) stress tolerance and molecular characterization of tolerant lines in sugarcane. BARC Newsletter Issue. no. 273:244-257.

Rao MVS, Kumari PK, Manga V, Mani NS, 2007. Molecular markers for screening salinity response in Sorghum. Indian Journal of Biotechnology. 6:274-276.

Sholihah NF, and Saputro TB, 2016. Response of Maize (*Zea mays* L.) var. Manding to Salinity Stress (NaCl) by In Vitro. Jurnal Sains dan Seni ITS. 3(2) : 2337-3520

Sukma KPW, Daryono BS, Purnomo, Suprapti I, 2018. Salinity resistance of seven varieties of Madura corn on early stage of growth. 4th International Conference on Food and Agriculture Resources (FANRes 2018). Atlantis Press : Advances in Engineering Research. 172: 181-184.

Xue D, Huang Y, Zhang X, Wei K, Westcott S, Li C, Chen M, Zhang G, Lance R, 2009. Identification of QTLs associated with salinity tolerance at late growth stage in barley. Euphytica. 169(2) : 187-196.

Yang Q, Jian-guo H, Yan S, Ming-sheng W, 2005. Identification of molecular marker linked to salt tolerance gene in alfalfa. Agricultural Sciences in China. 4: 22-29.

Younis-Rania AA, Ahmed MF, Mervat M, Menshawy EL, 2007. Molecular genetic markers associated with salt tolerance in grain sorghum. Arab J. Biotech. 10(2):249-258.