Agrotech Journal
ATJ
Url: http://usnsj.com/index.php/ATJ
Email: editor.atj@usnsj.com

Selection of Chilli Pepper (*Capsicum frutescens* L.) for Salinity Tolerance in Seed Germination

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ARTICLE INFO

e-ISSN: 2548-5148
p-ISSN: 2548-5121
Vol. 4, No. 2, December 2019
URL : http://dx.doi.org/10.31327/atj.v4i2.1110

Abstract

The obstacle of chilli pepper development in saline is that there is no salinity tolerant variety, so it is necessary to assemble tolerant varieties. Information on tolerant genotypes, selection criteria and determination of new selection methods at the germination level are needed to make it easier for breeders to select prospective tolerant varieties early. This study aims to determine tolerant genotypes, appropriate selection criteria and NaCl concentrations used for selection at the germination level. The study was arranged based on a completely randomized design with two factors: NaCl concentration and some chilli pepper genotypes. NaCl solution concentrations consist of five concentrations of N0: 0 g L^{-1} (EC 291 µS/cm, SAL 0,10), N1: 2 g L^{-1} (EC 3,71 ms/cm, SAL 2,0), N2: 4 g L^{-1}(EC 6,60 mS/cm, SAL 3,60), N3: 6 g L^{-1} (EC 9,56 mS/cm, SAL 5,40) and N4: 8 g L^{-1} (EC 12,45 mS/cm SAL 5,40). The second factor is the genotype of chilli pepper consisting of 22 genotypes. The results showed that the most tolerant genotypes were G4, G7 and G15. Characters that can be used as selection criteria at the germination level are the percentage of germination, radical and hypocotyl length. The concentration of 8 g NaCl L^{-1} is effectively used to select tolerant genotypes

Keywords: chilli pepper, germination, tolerant, salinity, selection

A. Introduction

Chilli Pepper (*Capsicum frutescens* L.) is one of the vegetable commodities that has a high economic value, because of its large enough role to meet domestic needs, export commodities and the food industry (Hartuti & Sinaga 1997), so that it included in one product that is quite strategic. In certain seasons, chilli pepper prices are significant enough to affect inflation. This price fluctuation occurs almost every year and is disturbing the public. The government's effort to overcome the change of chilli pepper prices is an effort to increase the chilli pepper planting area and stabilize the price of chilli pepper. Chilli pepper needs for large cities with a population of one million or more around 66,000 tons/month. In the festive season or religious holidays, the need for chilli pepper usually increases by about 10-20% of regular needs. The level of national chilli pepper productivity in 2015 was around 7.49 tons/ha. To supply the needs of chilli pepper for urban communities, a chilli pepper harvest area of about 11,000 ha/month is needed. Not to mention the pepper needs for rural towns or small cities and processed raw materials (Pusdatin, 2016).

Chilli pepper production, according to Saptana, Daryanto, Daryanto & Kuntjoro, (2010), is influenced by productivity and area of arable land. The chilli pepper harvested area for the period 2011-2015 tended to increase with an average growth of 5.54%. The growth of the planted area in Java was 6.87% while outside Java, it was 4.07% (Pusdatin, 2016). The growth of the harvested area has not been able to overcome the shortage of chilli pepper supply, so it is still very much needed to increase the area and increase the productivity of higher chilli pepper.

Chilli pepper is included in the group of plants that are sensitive to salinity (Lessani & Marscher, 1978). Efforts that can be made to increase the productivity of chillies in saline land are by using salinity tolerant superior varieties. Superior varieties are one of the factors that influence the success of production (Syukur, Sujiprihati, Yunianti, & Kusumah, 2010). At present, there are no commercially produced lines or varieties of commercial chilli pepper tolerance. Types that are tolerant to environmental stress in generally have low production, and conversely, superior varieties are usually more productive if planted on fertile land. In tomato plants, salinity tolerant varieties are wild varieties that are used as elders as a source of tolerance (Saranga, Cahaner, Zamir, Marani, & Rudic, 1992). According to Kusumainderawati, Retnaningtyas, Suwarno, Sugiartini & SUnaryo (2001), chilli pepper lines and superior hybrid varieties are more productive on fertile and sufficiently irrigated lands. Therefore, to increase the productivity of chilli pepper in saline land, it is necessary to assemble types of chilli pepper that are tolerant of salinity stress and high yields through hybridization between saline tolerant parents and high yield parents.

Assembling salinity tolerant varieties requires the existence of donor parents who have the tolerance to salinity and information about genetic control (gene action). Tolerance of environmental stress is a quantitative character with complex genetic phenotypes and controls. Basic understanding of genetic tolerance in plants is a prerequisite for developing superior genotypes. Alia, Baihaki, Hermiati, & Yuwariah. (2004) state that the pattern of inheritance, genetic variability and heritability of a character are genetic parameters related to the selection process and the incorporation of essential characteristics in a genotype. Sekesi in the initial stages of germination will facilitate further completion. Salinity selection at the germination level is expected to make efficient the stages of the selection method used. Accuracy in determining the selection method will largely determine the success of plant assembly.

B. Methodology

This experiment was arranged based on a Completely Randomized Design with two factors: NaCl concentration and several chilli pepper genotypes. NaCl solution concentration consists of 5 levels that modified from Kaouther, Elouer, Aloui, & Hannachi (2012) and Kaouther, Nina, Rezwan, & Cherif (2013) research results of namely 0 g L^{-1} (EC 291 µS/cm, SAL 0,10), 2 g L^{-1} (EC 3,71 ms/cm, SAL 2,0), 4 g L^{-1}(EC 6,60 mS/cm, SAL 3,60), 6 g L^{-1} (EC 9,56 mS/cm, SAL 5,40) and 8 g L^{-1} (EC 12,45 mS/cm SAL 5,40). EC and SAL was obtaining from media analysis according to NaCl concentration.

The second factor is the genotype of chilli pepper consisting of 22 genotypes. The experiment consisted of three replications, each unit consisting of one petri dish containing 20 seeds.

A total of 20 seeds per genotype were germination until a radicle (± 1 mm) appeared. The sprouts were growing in Petri dishes containing three layers of saturated opaque paper NaCl solution according to the treatment. Control as a comparison only saturated with Aquadest as the optimal germination media. The procedure gave for 14 days.

Petri dishes containing sprouts were incubating at room temperature (27-30^{o}C). Variables observed were vigour index (calculated as percen of germination, which grew normally strong and synchronously; radicle and hypocotyl length and fresh weight at 14 days after treatment (DAT). Based on these data, a reduced concentration of 50 (RC50) will be calculated, which is the concentration of NaCl which can cause growth inhibitions up to 50%. The observational data analysed with the F test variance. If the difference showed a significant effect at 5% level followed by the Duncan Multiple Range Test (DMRT) using the SAS 9.1 test facility.

C. Results and Discussion

Seed germination is one of several criteria that can be used to tolerate salinity stress. Increased salinity stress decreases the percentage of germination in Tomato, cucumber, shallots and chilli plants (Kurniasih & Toekidjo, 2008; Bojovic, Gorica, Marina, & Milan, 2010; Abari, Nasr, Hojjati, & Bayat 2011; Hassen, Nina, Rezwan, & Cherif, 2014). Bojovic *et al*. (2010) explain that salinity stress can primarily reduce the percentage of germination and delay the emergence of sprouts.

Tabel 1. Per sen Germination

Genotype	Concentration of NaCl							average		DMRT G	DMRT N
	N0		N1	N2	N3	N4					
G1	100,00	a	33,33	11,11	22,22	0,00		33,33		5,972	2,847
G2	100,00	a	91,67	72,22	69,45	41,67		75,00		6,662	3,176
G3	100,00	a	75,00	80,56	75,00	33,33		72,78		6,295	3,001
G4	100,00	a	91,67	91,67	75,00	86,11	a	88,89	a	6,511	3,104
G5	100,00	a	66,67	75,00	66,67	75,00	b	76,67		6,791	3,238
G6	100,00	a	52,78	88,89	86,11	66,67	c	78,89		6,877	
G7	100,00	a	83,33	83,33	83,33	63,89		82,78	a	6,964	
G8	97,22	a	41,67	11,11	5,55	0,00		31,11		7,028	
G9	100,00	a	50,00	50,00	58,33	50,00		61,67		7,093	
G10	100,00	a	63,89	77,78	58,33	36,11		67,22		7,201	
G11	100,00	a	83,33	58,33	58,33	41,67		68,33		7,201	
G12	100,00	a	91,67	86,11	91,67	61,11		86,11	a	7,244	
G13	100,00	a	97,22	83,33	83,33	47,22		82,22	a	7,287	
G14	100,00	a	33,33	5,55	0,00	0,00		27,78			
G15	100,00	a	94,45	66,67	75,00	91,67	a	85,56	a		
G16	97,22	a	19,45	5,55	0,00	0,00		24,44			
G17	100,00	a	66,67	83,33	25,00	0,00		55,00			
G18	100,00	a	88,89	100,00	77,78	55,55		84,44	a		
G19	100,00	a	83,33	86,11	0,00	16,67		57,22			
G21	100,00	a	58,33	36,11	16,67	0,00		42,22			
G25	94,44	a	77,78	69,45	58,33	72,22	bc	74,44			
G31	100,00	a	100,00	66,67	75,00	55,56		79,44	b		
Average	99,50 a		70,20 b	63,13 c	52,78 d	40,66 e		65,25			

Increasing salt concentration will inhibit water absorption by plant roots, where plants need water for photosynthesis, absorption of nutrients and other metabolic processes in plants so that increasing salt concentration will impede plant growth. The mechanism of inhibition of germination and growth of nurseries with NaCl is related to inadequate water absorption or may be derived from toxic effects on embryos (Azza, El-Quesni, & Farahat, 2007).

Table 1 shows that at concentrations of NaCl 0 grams (without the addition of NaCl in germination media), the average number of germinated seeds for all genotypes did not differ where 100 percent of sprouts grew except in G8, G16 and G25. At a concentration of 8 grams of NaCl per Liter (N4), the most germinating genotypes were G4 and G15, and differed from other genotypes based on the Duncan multiple range test. The difference in the level of salinity stress has a very significant effect on the percentage and time of germination (Zhani, Elouer, Aloui, & Hannachi 2018).

Tabel 2. Radicle Length

Genotype	Concentration of NaCl							average		DMRT G	DMRT N
	N0		N1	N2	N3	N4					
G1	3,42		2,77	3,34	0,88	0,69		2,22	c	0,433	0,206
G2	4,49	ab	4,30	2,05	2,05	0,00		2,58	ab	0,456	0,217
G3	3,54		2,89	2,05	1,81	1,49	bc	2,35	b	0,472	0,225
G4	3,40		2,92	2,55	1,77	2,44	a	2,62	ab	0,483	0,230
G5	3,87		2,90	1,49	1,05	0,91		2,04		0,492	
G6	3,77		2,52	1,60	1,63	1,35	c	2,18		0,498	
G7	4,55	ab	3,47	2,06	2,31	1,55	bc	2,79	ab	0,504	
G8	1,23		1,08	1,13	1,90	1,22	d	1,31		0,509	
G9	3,08		3,25	2,24	0,28	0,00		1,77		0,514	
G10	4,24	b	2,84	1,84	1,48	1,36	c	2,35	b	0,522	
G11	1,77		1,88	1,63	0,80	0,97		1,41		0,522	
G12	3,59		3,21	2,32	2,22	1,84	bc	2,64	ab	0,525	
G13	3,88		2,81	1,75	1,72	1,38	c	2,31	b	0,528	
G14	4,03	c	2,53	1,30	2,93	1,05		2,37	b		
G15	4,79	a	4,30	2,11	1,54	0,00		2,55	ab		
G16	1,73		3,55	1,00	3,02	0,94		2,05			
G17	4,31	b	2,55	0,24	0,00	0,00		1,42			
G18	2,92		1,71	1,67	0,00	0,00		1,26			
G19	3,95		2,63	2,02	1,97	1,17		2,35	b		
G21	4,16	b	4,74	1,75	0,00	0,19		2,17			
G25	2,92		2,66	2,25	1,59	0,00		1,88			
G31	2,98		4,23	3,89	1,70	1,89	b	2,94	a		
Average	3,48 a		2,99b	1,92c	1,48d	0,93e					

Based on the average amount of germination at all concentrations of NaCl, genotypes G4, G7, G12, G13, G15 and G18 have higher germination percentages and are significantly different from other genotypes. G7, G12, G13 and G18 have the average number of high germination for all concentrations but are markedly different from the highest genotype at the highest NaCl concentration (N4) (Table 1). The response of various cayenne peppers to salinity differs between genotypes (Niu & Rodriguez, 2010). The salinity treatment showed a real influence on the germination parameters, i.e. the percentage of hypocotyl length germination and germination root. Water containing NaCl is an external factor that has a dominant role against germination. In chilli, decreasing salinity stress loses the percentage of germination (Bojovic *et al*., 2010; Abari *et al*., 2011).

Tabel 3. Hypocotil length

Genotype	Concentration of NaCl							average		DMRT	DMRT
	N0		N1	N2	N3	N4				G	N
G1	4,17	a	3,55	4,67	1,14	2,30	cd	3,16	a	0,383	0,183
G2	3,20	d	3,85	2,30	1,86	0,00		2,24		0,404	0,192
G3	3,88	ab	3,46	2,34	1,96	2,40	cd	2,81	abc	0,418	0,199
G4	3,76	ab	1,31	0,70	0,18	3,20	a	1,83		0,427	0,204
G5	2,70		2,39	1,47	1,23	1,35		1,83		0,436	0,208
G6	2,54		2,59	1,76	1,70	0,97		1,91		0,441	0,210
G7	3,08		2,97	2,03	1,48	1,82		2,27	d	0,447	0,213
G8	2,18		1,50	0,76	1,11	1,37		1,38		0,451	0,215
G9	3,15		3,25	2,89	0,68	0,00		1,99		0,455	0,217
G10	3,18		2,62	2,08	1,52	1,55		2,19		0,462	0,220
G11	2,23		2,73	2,97	2,11	1,92	e	2,39	c	0,462	0,220
G12	2,92		2,98	2,16	1,90	2,29	cd	2,45	c	0,465	0,221
G13	2,25		1,74	1,58	0,00	0,00		1,11		0,467	0,223
G14	3,22	d	3,20	2,46	2,13	2,67	bc	2,73	bc		
G15	3,55	bc	3,51	2,30	3,25	3,00	ab	3,12	ab		
G16	2,15		1,73	0,77	1,66	1,18		1,50			
G17	3,61	bc	2,59	1,90	0,00	0,00		1,62			
G18	3,65	bc	4,25	2,72	2,04	0,00		2,53	c		
G19	3,18		2,95	2,17	2,24	2,11	d	2,53	c		
G21	2,81		3,41	1,33	0,00	0,53		1,62			
G25	3,28	c	3,07	2,06	1,43	0,00		1,97			
G31	3,71	b	2,88	1,92	2,17	1,54		2,44	c		
average	3,11 a		2,84 b	2,06 c	1,44 d	1,37 d					

At 8 grams NaCl concentration (N4) the average percentage of seed germinated below 50 percent so that this concentration can be used as an effective concentration for the selection of chilli pepper to salinity at the germination level.

The longest radicles at an average of all concentrations are G2, G4, G7, G12, G18, and G31. The genotypes with the longest radicles at 0 g L-1 (N0) NaCl concentrations were G2, G7, and G15. At the highest NaCl concentration of 8 g L-1 (N4), the longest radicle genotype was G4 and significantly different from the other genotypes (Table 2). Plant tolerance to salinity stress is different for each variety (Aini, Mapfumo, Rengel, & Tang 2012).

Tolerant plants have better radicular length compared to sensitive plants. The mechanism of growth inhibition and seedling growth with NaCl may be related to the radicles due to insufficient air movement or may cause toxic effects on the embryo (Azza *et al.,* 2007). The results of the study by Katsuhara & Kawasaki, (1996) stated that research on root growth in salt stress is caused by turgor pressure for cell growth because the osmotic potential of the media grows lower than the osmotic potential in cells, and salt cells. The limited information on salinity tolerant chilli varieties is the basis of research, so the results of the study will get a variety of salinity tolerant plants and in the future how to increase crop production in saline land.

Tabel 4. Fresh Weight of sprouth

Genotype	Concentration of NaCl							average		DMRT G	DMRT N
	N0		N1	N2	N3	N4					
G1	5,09		3,57	3,11	2,76	2,15	e	3,33		0,204	0,097
G2	4,88		5,34	4,28	3,21	1,36		3,81	d	0,215	0,102
G3	6,10		5,80	3,87	1,52	2,30	d	3,92	d	0,222	0,106
G4	7,01	de	5,80	4,58	3,52	3,06	a	4,79	a	0,227	0,108
G5	4,88		3,67	3,26	1,84	2,45	d	3,22		0,232	
G6	6,40	g	4,12	3,36	2,50	2,76	bc	3,83	d	0,235	
G7	8,28	b	4,68	4,62	3,78	2,35	d			0,238	
G8	1,54		1,08	1,54	4,28	2,60	cd	2,21		0,240	
G9	2,00		5,80	2,12	2,42	1,30		2,73		0,242	
G10	5,04		4,83	4,23	3,36	2,30	d	3,95	d	0,246	
G11	6,10		6,25	2,15	4,12	2,76	bc	4,28	b	0,246	
G12	6,86	ef	4,15	2,67	1,44	1,24		3,27		0,247	
G13	7,16	d	4,28	3,21	2,45	2,91	ab	4,00	cd	0,249	
G14	1,84		3,77	2,30	2,30	1,84		2,41			
G15	8,68	a	5,64	3,82	3,36	2,91	ab	4,88	a		
G16	2,60		2,76	2,45	2,28	2,00		2,42			
G17	4,58		4,43	3,00	1,47	1,60		3,02			
G18	6,58	fg	6,40	3,97	2,45	1,45		4,17	bc		
G19	6,20		5,19	3,67	1,84	1,54		3,69	e		
G21	6,25		5,19	2,35	1,36	0,95		3,22			
G25	7,77	c	5,85	3,36	1,98	0,60		3,91	d		
G31	2,15		3,67	2,24	2,91	2,45	d	2,68	a		
Average	5,36 a		4,65 b	3,19 c	2,60 d	2,04 e					

The results of hypocotyl length in Table 3 show that the genotypes with the longest hypocotyl are G1, G3, and G4 in no add NaCl concentration (N0). Based on the average for all levels, the genotype with the longest hypocotyl is the genotypes G1, G3 and G15. At the high NaCl concentration of 8 grams L^{-1} (N4), the genotypes that had the longest hypocotyl were G4, and G15 (Table 3). Salinity hurts the growth of chilli plants caused by osmotic stress and the toxicity of sodium chloride. Salt stress strengthens the parameters of germination, root length and plamula (Hassen, Maher & Cherif, 2014; 2004; Khan, Ayub, Pervez, Bilal, Shahid, & Ziaf, 2009).

More tolerant genotypes will promote better growth at high-stress levels than sensitive genotypes that support starting growth compared to the increased concentration of NaCl given. Fresh weight of sprouts is one indicator of the growth of better germs. NaCl (N0) is G15 and significantly different from other genotypes. The genotypes with the best average fresh weight of sprouts at all concentrations were G4, and G15. As for the highest NaCl concentration (N4), the best fresh weight is the genotypes G4, G13 and G15 (Table 4). Genotype with good germination will produce good plant growth too (Nerson, 2007). The results of the research of Siregar Rosmayati, & Julita (2010) reported that administration of 0-750 ppm NaCl did not show any significant difference at the beginning of the nursery, but supported a considerable increase in height after being added to 400 ppm NaCl at planting. It is indicated that germination mostly determines the initial growth of plants. If germination growth inhibited, then further growth will also be frustrated. Therefore salinity must be selected which starts at the initial stage of germination

D. Conclusions

The results showed that the most tolerant genotypes were G10 and G12. Characters that can be used as selection criteria at the germination level are the percentage of germination, radical and hypocotyl length. The concentration of 8 g NaCl L-1 is effectively used to select tolerant genotypes.

E. Acknowledgments

This study was financially supported by the Directorate General of Higher Education, Ministry of National Education of Indonesia, which has provided funding through the Insinas Grant (Insinas Riset Pramata Individu) in 2019 on behalf of Marlina Mustafa with the project number 39/INS-1/PPK/E4/2019.

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