III.3 DEVELOPMENT OF SOME DIHAPLIOD RICE LINES UNDER SALINITY AND WATER DEFICIT CONDITIONS USING ANTHER CULTURE TECHNIQUE

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Abstract

Anther culture technique was utilized to produce doubled-haploid rice lines (DHLs) tolerant to soil stresses, especially drought and salinity. The introduced tolerant Korean rice cultivar RyongBuk 10 was crossed with the Egyptian sensitive cultivar Sakha 102 during 2007 season. The anthers of resulting F_1 were plated on N6 (callus induction media) and regenerated on MS (plant regeneration media) to produce DHLs. Forty five DHLs were obtained, out of them two were superior and selected for further study. The two parents, i.e., Ryong Buk 10 and Sakha 102, the two selected DHLs and the local check Giza 178 were evaluated in the field under normal, water deficit, and saline soil conditions during the two rice summer seasons of 2012 and 2013. Fifteen vegetative yield and yield component traits were measured for all studied genotypes. Significant and highly significant differences were observed among studied genotypes for all studied traits. The two developed DHLs, AC 1 and AC 2, showed the highest and most favorable values of mean performance for all studied traits and were superior to their two respective parents as well as Giza 178 cultivar. AC 1 and AC 2 scored the highest grain yield over their Egyptian parent Sakha 102 by 73.6% and 63.4%, respectively, under water deficit condition, while both DHLs recorded high grain yield advantage over Sakha 102 by 54.9% and 51.4% under saline soil condition.

Keywords: *Oryza sativa* L., doubled haploid, soil stress, drought, salinity, varietal improvement.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops all over the world as well as in Egypt. Great efforts have been done to develop high yielding and stress tolerant modern Egyptian rice varieties. As a result of these efforts, Egypt has now many highly stable yielding varieties. In spite of that, no Egyptian rice cultivar could be nominated as highly tolerant to water deficit and salinity stresses, which represent the two major constraints of rice cultivation in Egypt. So that, great efforts should be paid to develop breeding programs to achieve Egyptian water deficit and salinity tolerant rice varieties.

Rice is a semi-aquatic plant and very sensitive to water deficit (El-Hessewy, 2002 and Lafitte *et al.*, 2007).The main constraint of rice cultivation in Egypt is the limited source of irrigation water from Nile River and the shortage of available water, especially in canal's terminals in North Delta, whichfacing the increased levels of salinity for soil and irrigation water. The modern tools of biotechnology can help rice breeders to accelerate the breeding cycle to develop new rice lines, and save long periods of time, high expenses and great efforts needed for classical breeding methods (Elmoghazy, 2007).

Tissue culture, especially anther culture, represents one of the most valuable biotechnological tools for accelerating rice breeding (Draz, 2002 and Gioi and Tuan, 2004).The anther culture technique was first developed in rice by Niizeki and Oono (1968). The Rice Anther Culture Lab. at Rice Research and Training Center (RRTC) in Egypt was established by Prof. Abdelsalam Draz at 1992. This technique produces completely stabilized and uniform Doubled-Haploid Lines (DHLs) which bypass the inbreeding process (Brar and Khush 2006 and Germana 2011).It is the fastest method for DHLs production as it only takes between 8 and 9 months (Agache *et al.*, 1989). This technique manipulates the male sex cells in immature anthers, to induce haploid callus formation, which are spontaneously converted to double haploid embryos (Niizeki and Oono, 1968). Genetic recombination occurs during haploid sex cell production, so that each microspore (immature pollen) which is produced is genetically unique. Consequently, each DHL obtained in this way will produce a new stabilized and unique line. This breeding tool has been used not only to establish parental pure lines saving the long inbreeding process, but also to speed up descendant's selection after an artificial cross, bypassing the classical pedigree selection process (Courtois, 1993; Martinez *et al.*, 1996 and Moon *et al.*, 2003).

Anther culture technique was used extensively for varietal improvement in rice by many researchers i.e. Jiang *et al.* (2003), Abdel Maksoud *et al.* (2007), Sah and Niroula (2007), Purwoko *et al.* (2010), Xa and Lang (2011) and Serrat *et al.* (2014).

The objective of this study was to develop and evaluate some doubled-haploid rice lines tolerant to soil stress conditions especially water deficit and salinity and to focus on the importance of using anther culture technique in varietal improvement in rice.

MATERIALS AND METHODS

The present study was carried out at Rice Biotechnology Lab. and Rice Experimental Farm of Rice Research and Training Center (RRTC) and Elsirw rice research station field(as a saline soil condition) during the two successive summer seasons 2012 and 2013.

Plant materials

The introduced Korean rice cultivar Ryong buk 10 (tolerant to water deficit and salinity stress) was crossed with the Egyptian rice cultivar Sakha 102 (sensitive to water deficit and salinity stress) during summer season 2007. The resulting F_1 seeds were planted at summer season of 2008 as F_1 plants. Boots were collected from F_1 plants to practice anther culture technique.

Development of doubled-haploid lines(DHLs)

The anthers were plated at Rice Biotechnology Lab according to the anther culture technique described by Jiang *et al.* (2002) with some modifications as follows: Panicles of F_1 plants at reproductive stage were collected from primary tillers when the distance of the flag leaf auricle to that of the next leaf is about five to ten cm. This would approximate the mid uninucleate to early binucleate stages of pollen development. The boots or panicles were washed thoroughly with tap water, and then wrapped in towel paper moistened with distilled water. The boots were incubated in the dark at 8°C for eight days. Panicles were sprayed with 70% ethanol. After that, panicles were surface sterilized with 20% Clorox (Sodium hypochlorite 5.25%) for 20 min and were rinsed three times with sterilized distilled water. The basal part of the spikelets was cut out using sterilized scissors with ethanol and flam. Then anthers were excised with sterilized forceps from the spikelets in the middle of the panicle. About 100 anthers were inoculated in Petri dish (60 x 15 mm) containing ten ml of N6 media (Chu *et al.*, 1975). The dishes were sealed with Parafilm and incubated in darkness at 25±1°C. For plant regeneration, the calli formed 2-3 mm in diameters (collected after being incubated 1-2 months) were transplanted to Petri dishes containing plant regeneration MS medium (Murashing and Skoog, 1962) and kept in the light (16 hours light and 8 hours dark) at 25-27°C. Shoots developed and roots establishment are usually done in regeneration media in big test tubes (25 x 200 ml). Plantlets with vigorous rooting system were transferred to the culture solution for two weeks under normal day length for their adaptation to field conditions. Plantlets were individualized and replanted in the culture solution for one more week. After that, plantlets were finally transferred in pots (filled with Petmos 50% and clay 50%) to the greenhouse.

Anther culture derived plants, at beginning of flowering and before the onset of anthers, were bagged to prevent cross-pollination with other rice plants. Panicles were separately collected from the anther culture derived plants and forty-five spontaneous doubled haploid lines (DHLs) were obtained. These lines were planted and evaluated at rice planting seasons 2009, 2010 and 2011 to study the performance and stability of lines. Two lines of them, AC 2882 (abbreviated as AC 1) and AC 2884 (abbreviated as AC 2), were superior and selected for further evaluation studies along with their two respective parents, Ryong buk 10 and Sakha 102 as well as Giza 178 as the best Egyptian commercial rice cultivar under water deficit and salinity conditions (RRTC, 2001 and Elmoghazy, 2007), during 2012 and 2013 rice planting seasons.

Field evaluation

All genotypes, *i.e.*, the two DHLs AC 1 and AC 2, the parents Ryong buk 10 and Sakha 102, and Giza 178 were evaluated under three soil conditions at research field of RRTC and Elsirw rice research field, during the two summer seasons 2012 and 2013. The seedlings of all materials were divided into three parts. The first part was evaluated under normal irrigation conditions of continuous flooding as control (C). The second part was evaluated under water deficit conditions at Upland Nursery of RRTC with flush irrigation every 14 days without any standing water(D). The third part was evaluated at Elsirw rice research field (EC from 10 to16 dS m^{-1}), as saline soil (S). All materials were planted in randomized complete block design and replicated three times with twelve rows for each sample and 20 x 20 cm distance between rows and hills and five meters long. The ten internal rows were used in data collection and harvested for the yield to avoid border effect.The package of all other recommendations of rice planting was followed.

Measured characteristics

Fifteen different vegetative and yield and its component traits were measured for all studied genotypes. Forshoot characteristics, plant height (Ht), days to heading (DH), tillers $plant^{-1}$ (TiP) and shoot dry weight (SDW) were studied. For root characteristics, roots $plant^{-1}$ (RP), maximum root length (MRL), root thickness (RTh), root dry weight (RDW) androot to shoot ratio (RSR) were studied. For yield and its components, panicles $plant^{-1}$ (PnP), spikelets $panicle^{-1}$ (SPn), panicle length (PnL), spikelet fertility percent (SFP), thousand-grain weight (TGW) andgrain yield (g) per m^2, (GY m^{-2}) were studied.

Analysis of variance

The analysis of variance and expected mean squares of the studied characteristics for DHLs, their two parents and Giza 178 were computed using MSTAT statistical program with two factors randomized complete block design.

RESULTS AND DISCUSSION

The phenotypic acceptability, insect infection and disease susceptibility for all studied genotypes are illustrated in Table 1. Over all, the studied genotypes showed good phenotypic acceptability (according to Standard Evaluation System, SYS of IRRI, 2002) under different soil conditions, except for Sakha 102 under soil stress conditions.

The two developed AC lines showed excellent phenotypic acceptability under different soil conditions and were superior to their two parents and Giza 178 for almost all studied traits. All studied genotypes showed moderate susceptibility to brown spot, except for the two DHLs which were resistant. All studied genotypes showed highly resistance to blast disease.

Table 1. Phenotypic acceptability, insect infection and disease susceptibility for studied genotypes under control (C), Water deficit (D) and Salinity (S) conditions.

		Ryong Buk10	Sakha 102	Giza 178	AC 1	AC 2
Phenotypic acceptability[1]	C	3	3	2	1	2
	D	4	5	3	2	2
	S	4	6	4	3	3
Leaf minors (%)	C	6	7	9	3	3
	D	4	3	4	2	2
	S	3	3	4	4	4
Stem borers (%)	C	5	3	9	2	2
	D	4	2	3	2	3
	S	4	3	5	3	3
Brown spot[2]	C	3	4	4	1	1
	D	6	6	5	2	2
	S	5	5	4	2	2
Blast[2] (leaf and neck)	C	1	1	1	1	1
	D	1	1	1	1	1
	S	1	1	1	1	1

According to Standard Evaluation System (SYS) of IRRI,2002.

[1], scale from 1 for good acceptance to 9 for bad acceptance.

[2], scale from 1 for highly resistance to 9 for highly susceptible.

Almost all studied traits showed different degrees of reduced estimates under both soil stress conditions of water deficit and salinity for all studied genotypes with different degrees of tolerance for Ryong Buk 10, Giza 178 and the two DHLs. This result agreed with those obtained by other investigators for different genotypes (Abdel Maksoud *et al.*, 2007; Elmoghazy, 2007 and Purwoko *et al.*, 2010).

Shoot traits

Significant and highly significant variations were calculated among studied genotypes, treatments and interaction for shoot traits as shown in Table 2.The mean performance of shoot characteristics for studied genotypes is illustrated in Table 3. The two developed DHLs, AC 1 and AC 2 were superior to their two parents and Giza 178 for days to heading (DH), tillers plant^{-1} (TiP) and shoot dry weight (SDW) under the three soil conditions. AC 1 showed the highest estimates for TiP and SDW under all conditions followed by AC 2.

Table 2. Combined analysis of variance and the mean squares of genotypes, treatments, years and their interactions for shoot traits.

Source of variation	D.F.	Ht	DH	TiP	SDW
Years	1	48.77	23.95	345.1	181.69
Replications / years	4	0.07	0.09	285.0	52.69*
Treatments	2	6009**	1257**	279.3	319.99**
Years x treatments	2	96.19**	95.13**	243.1	263.14*
Genotypes	4	298.23**	160.65**	573.7**	693.39**
Genotypes x Years	4	22.06**	28.77**	283.4*	269.62**
Genotypes x treatments	8	36.77**	19.24**	621.3**	553.81**
Genotypes x treatments x years	8	8.76**	11.26**	633.2**	202.25**
Error	32	0.16	2.05	215.02	22.28

*, ** significant at 0.05 and 0.01 levels of probability, respectively. Abbreviations:Ht, Plant Height; DH, Days to heading; TiP, TillersPlant^{-1};SDW, Shoot Dry Weight.

Table 3. The mean performances of shoot characteristics for studied genotypes under control (C), Water deficit (D) and Salinity (S) conditions from the data combined over the two years 2012 and 2013.

Trait		Ryong Buk10	Sakha 102	Giza 178	AC 1	AC 2
Ht (cm)	C	115.05±0.45	110.23±0.47	97.01±0.25	111.50±0.43	112.08±0.33
	D	113.14±0.64	96.54±0.59	83.85±0.57	108.98±0.73	106.59±0.96
	S	108.02±0.78	83.36±0.85	78.08±0.98	97.76±0.88	96.05±0.99
DH (day)	C	98.05±0.08	96.01±0.11	108.02±0.12	94.66±0.20	95.23±0.08
	D	96.87±0.15	92.85±0.29	104.55±0.27	93.44±0.26	92.87±0.24
	S	99.08±0.42	98.92±0.40	106.33±0.36	95.66±0.42	96.02±0.38
TiP	C	17.55±0.04	18.24±0.05	22.36±0.07	24.21±0.02	23.29±0.02
	D	15.04±0.22	14.21±0.08	20.28±0.13	21.22±0.08	20.00±0.07
	S	14.53±0.34	11.02±0.11	16.04±0.18	17.22±0.15	16.72±0.12
SDW (g)	C	62.05±0.32	52.07±0.22	72.05±0.24	75.25±0.20	73.55±0.31
	D	55.46±0.54	37.28±0.63	58.34±0.49	60.02±0.48	58.91±0.68
	S	43.62±0.83	25.59±0.92	33.69±0.88	40.21±0.78	37.95±0.93

Abbreviations:Ht, Plant height (cm); DH, Days to heading; TiP, Tillersplant^{-1};

SDW, Shoot dry weight (g).

Root traits

The combined analysis of variance and the mean squares of genotypes, treatments, years and their interactions for root traits are represented in Table 4. Significant and highly significant estimates of variations were calculated among studied genotypes, treatments and interaction for root traits as shown in Table 4.

Table 4. Combined analysis of variance and the mean squares of genotypes, treatments, years and their interactions for roottraits.

Source of variation	D.F.	RP	MRL	RTh	RDW	RSR
Years	1	368.0	22.75*	0.004	2.19	0.157
Reps / years	4	298.1	0.103	0.005*	1.49	0.007
Treatments	2	394.0*	457.00**	0.007**	140.15**	0.260**
Years x treatments	2	223.2	97.22**	0.037**	11.98**	0.128**
Genotypes	4	458.4**	143.23**	0.701**	2940**	10.31**
Genotypes x Years	4	275.2	28.57**	0.052**	10.215**	0.392**
Genotypes x treatments	8	521.5**	20.16**	0.012**	15.344**	0.299**
Genotypes x treatments x years	8	578.3**	16.19**	0.014**	11.359**	0.357**
Error	32	226.2	3.07	0.001	0.052	0.008

*, ** significant at 0.05 and 0.01 levels of probability, respectively.Abbreviations:RP, rootPlant^{-1}; MRL, Maximum root length; RTh, Root thickness; RDW, Root dry weight; RSR, Root to shoot ratio.

Both DHLs showed the most favorable estimates and were superior to their two parents and Giza 178 for mean performance of root characteristics (Table 5). AC 1 recorded the highest estimates for roots plant^{-1} (RP), maximum root length (MRL), root thickness (RTh) and root dry weight (RDW) under the three soil conditions.

Table 5. The mean performances of root characteristics for studied genotypes under control (C), Water deficit (D) and Salinity (S) conditions from the data combined over the two years 2012 and 2013.

Trait		Ryong Buk10	Sakha 102	Giza 178	AC 1	AC 2
RP	C	193.21±4.5	139.66±3.2	182.93±5.5	215.24±4.3	217.23±5.3
	D	175.02±6.4	111.02±5.2	145.69±6.7	195.36±5.9	190.45±7.1
	S	143.54±8.7	89.36±6.8	115.96±7.6	154.37±6.8	146.87±7.3
MRL	C	31.42±0.24	21.35±0.18	26.35±0.28	35.58±0.25	33.53±0.24
	D	27.45±0.37	18.58±0.33	22.31±0.42	27.53±0.41	25.57±0.51
	S	19.85±0.48	15.02±0.42	18.52±0.57	22.11±0.50	21.10±0.52
RTh	C	0.87±0.02	0.54±0.01	0.58±0.01	0.91±0.01	0.81±0.01
	D	0.77±0.02	0.41±0.02	0.46±0.02	0.82±0.03	0.79±0.02
	S	0.43±0.03	0.34±0.02	0.40±0.03	0.74±0.03	0.71±0.03
RDW	C	6.31±0.04	3.75±0.03	5.84±0.02	6.89±0.02	6.75±0.02
	D	5.52±0.06	2.93±0.03	4.53±0.03	5.88±0.03	5.52±0.03
	S	4.71±0.05	2.21±0.04	3.61±0.03	4.30±0.04	4.01±0.04
RSR	C	0.102±0.002	0.071±0.001	0.082±0.002	0.093±0.002	0.085±0.002
	D	0.107±0.003	0.077±0.003	0.076±0.002	0.098±0.003	0.092±0.003
	S	0.108±0.005	0.086±0.003	0.107±0.004	0.108±0.004	0.101±0.004

Abbreviations:RP, root Plant^{-1}; MRL, Maximum root length (cm); RTh, Root thickness (mm); RDW, Root dry weight (g); RSR, Root to shoot ratio.

Yield and its components

The combined analysis of variance and the mean squares of genotypes, treatments, years and their interactions for yield and its component ttraits are presented in Table 6. As observed for vegetative traits, significant and highly significant estimates of variations were calculated among studied genotypes, treatments and interaction for yield and its componenttraits. The two developed DHLs, AC 1 and AC 2, showed the highest and most favorable mean performances for yield and its component traits over their two parents and Giza 178 as observed in Table 7. AC 1 showed the highest value for grain yield per m^2 under the three soil conditions with 1102.3, 698.8 and 523.6 g m^{-2} for C, D and S conditions, respectively. The two DHLs recorded also the most favorable estimates for panicles plant^{-1}; spikelets panicle^{-1}; panicle length, spiklelets fertility percent and thousand grain weights.

The Ryong Buk 10 parent showed the lowest grain yield reduction percent due to water deficit (32.4%) and salinity (37.6%) stress conditions, which could be due to its tolerance to soil stresses and lowest yield under normal condition (Table 8). Sakha 102 showed the highest grain yield reduction percent under water deficit (55.8%) and salinity (62.9%) conditions. Both DHLs, AC 1 and AC 2, showed lower grain yield reduction percent than their Egyptian parent Sakha 102 and the best local check Giza 178 under soil stress conditions (Table 8).

Table 6. Combined analysis of variance and the mean squares of genotypes, treatments, years and their interactions for yield and its componenttraits.

Source of variation	D.F	PnP	SPn	PnL	SFP	TGW	GY m^{-2}
Years	1	299	123.9*	376	33.3*	4.77	799.61
Reps / years	4	315	198.0	301	336.6*	26.16*	818.64*
Treatments	2	285**	159.1**	322*	286.5**	99.55**	847.50**
Years x treatments	2	203	193.2**	285*	105.5**	47.63**	842.30**
Genotypes	4	773**	169.2**	673**	355.5**	74.24**	7495.78**
Genotypes x Years	4	245	131.8**	293	605.8	35.63**	6093.84**
Genotypes x treatments	8	643**	119.2**	635**	350.6	129.65	9953.54**
Genotypes x treatments x years	8	672**	112.3**	662**	393.0**	58.85**	2522.18**
Error	32	219	107.03	228.1	10.50	0.35	66.24

* and ** significant at 0.05 and 0.01 levels of probability, respectively. Abbreviations:PnP, Panicles $Plant^{-1}$; SPn, $SpikeletsPanicle^{-1}$; PnL, Panicle Length; SFP, Spiklelets Fertility Percent; TGW, Thousand Grain weight; GY m^{-2}.

Table 7. The mean performances of yield and its component characteristics for studied genotypes under control (C), Water deficit (D) and Salinity (S) conditions from the data combined over the two years 2012 and 2013.

Trait		Ryong Buk10	Sakha 102	Giza 178	AC 1	AC 2
PnP	C	16.58±0.04	17.34±0.05	20.36±0.07	23.21±0.02	21.29±0.02
	D	14.04±0.12	12.21±0.07	19.28±0.12	20.25±0.08	19.02±0.08
	S	12.53±0.34	10.00±0.09	14.04±0.18	17.17±0.15	16.02±0.14
SPn	C	175.65±2.01	185.22±2.60	205.21±2.60	215.02±2.47	210.04±2.87
	D	155.21±3.20	149.34±3.47	183.02±3.14	193.08±3.65	186.05±3.77
	S	136.01±3.89	122.38±4.26	146.32±3.67	168.36±4.44	159.06±4.87
PnL	C	18.51±0.21	19.31±0.14	21.31±0.09	20.92±0.06	20.76±0.08
	D	18.20±0.30	18.10±0.21	20.01±0.11	20.10±0.12	19.91±0.13
	S	17.62±0.43	17.01±0.32	19.02±0.27	19.21±0.26	19.01±0.30
SFP	C	86.36±1.31	89.67±1.24	82.13±1.81	93.24±1.56	92.39±1.25
	D	78.68±2.35	70.58±2.35	74.25±2.21	82.27±2.31	80.55±2.87
	S	74.66±3.24	68.12±3.76	71.21±3.24	77.93±3.33	74.96±3.57
TGW	C	22.94±0.08	24.25±0.07	19.82±0.15	26.96±0.07	25.57±0.09
	D	20.32±0.12	21.68±0.09	17.10±0.21	24.85±0.16	24.25±0.18
	S	19.82±0.14	20.95±0.14	16.58±0.22	22.76±0.21	21.44±0.22
GY m^{-2}	C	705.32±10.1	911.42±10.3	976.38±11.1	1102.3±11.8	1095.2±10.7
	D	476.55±12.3	402.46±11.2	501.22±12.2	698.85±17.5	657.54±13.8
	S	439.88±22.5	338.11±21.3	411.28±18.5	523.59±18.9	511.76±17.9

Abbreviations:PnP, Panicles Plant^{-1}; SPn, SpikeletsPanicle^{-1}; PnL, Panicle Length (cm); SFP, Spiklelets Fertility Percent; TGW, Thousand Grain weight (g); Gy m^{-2}.

Table 8. The grain yield reduction percent due to soil stress conditions, Water deficit (D) and Salinity (S).

Stress	Ryong Buk10	Sakha 102	Giza 178	AC 1	AC 2
D	32.43	55.84	48.67	36.60	39.96
S	37.63	62.90	57.88	52.50	53.27

As observed in Table 9 the two DHLs recorded high yield advantages over their two parents and the best local check cultivar Giza 178 under the three different soil conditions. AC 1 and AC 2 scored the highest grain yield advantages over its Egyptian parent Sakha 102,73.6% and 63.4%, respectively, under water deficit condition. Also both DHLs recorded high yield advantages over Sakha 102 (54.9% and 51.4%) under saline soil condition.

Table 9. The grain yield advantages (as a percentage) of AC 1 and AC 2 over their two parents and Giza 178 under different soil conditions.

		Ryong Buk10	Sakha 102	Giza 178
AC 1	C	56.28	20.94	12.89
	D	46.65	73.64	39.43
	S	19.03	54.86	27.31
AC 2	C	55.28	20.16	12.17
	D	37.98	63.38	31.19
	S	16.34	51.36	24.43

It could be recommended that, anther culture technique represents a powerful tool for accelerating varietal improvement in rice. Also, AC 1 line strongly recommended to be registered as the first Egyptian rice cultivar tolerant to soil stress conditions, especially water deficit and salinity, also early and blast resistant, developed through anther culture technique in Egypt.

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٣-٣ استنباط سلالات أرز متضاعفة كروموسوميا تحت ظروف الملوحة ونقص المياه باستخدام تقنية زراعةالمتوك

أشـــــرف محـــمـــدالـــمـــغـــــــــازي

قسم بحوث الأرز – معهد بحوث المحاصيل الحقلية – مركزالبحوث الزراعية – الجيزة – مصر.

تم الاستفادة من تقنية زراعة المتوك لإنتاج سلالات أرز أحادية متضاعفة (DHLs) متحملة لإجهادات التربة خاصة الجفاف والملوحة. حيث تم تهجين الصنف الكوري المستورد المتحمل RyongBuk 10 مع الصنف المصري الحساس سخا ١٠٢. وتم استزراع متوك نباتات الجيل الأول الناتج على بيئة N6 ونقل الكالوس المتكون على بيئة MS لإنتاج عدد من الـ DHLs. وقد تم إنتاج ٤٥ سلالة، كانت اثنتان منها متفوقة وتم انتخابها لمزيد من الدراسة. تم تقييم كلا الأبوين RyongBuk 10 و سخا ١٠٢ والسلالتين الـ DHLs وكذلك الصنف المصري جيزة ١٧٨ حقليا تحت الظروف الطبيعية والجفاف والملوحة خلال موسمي الزراعة ٢٠١٢ و ٢٠١٣. وقد درست ١٥ صفة خضرية ومحصولية لجميع التراكيب الوراثية المدروسة. وأوضحت دراسة التباين درجة تباين معنويه وعالية المعنوية بين التراكيب الوراثية والمعاملات والتفاعل لكل الصفات المدروسة. كما أظهرت سلالتي الـ DHLs : AC2 ،AC 1 أفضل قيم لمتوسط الشكل الظاهري لكل الصفات التي تم دراستها وكانت متفوقة على كلا أبويها وكذلك جيزة ١٧٨. وقد سجلت كلتا السلالتين AC 1 و AC 2 تفوقا في محصول الحبوب على الأب سخا ١٠٢ بمقدار ٧٣.٦% و ٦٣.٤%، على التوالي تحت ظروف الجفاف. كما أظهرتا تفوقا في المحصول بنحو ٥٤.٩% و ٥١.٤% على سخا ١٠٢ تحت ظروف الأرض الملحية.