Genetic Analysis of Cadmium Tolerance And Exploring Its Inheritance Nature In Bread Wheat

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Abstract

Cadmium (Cd) is a nonessential and extremely toxic element that destructively impacts agricultural production. Accordingly, developing tolerant-Cd as well as low-grain Cd genotypes is considered a promising approach to cope with the pollution problem. The current study aimed at understanding inheritance nature of Cd tolerance and detect Cd-tolerant and low-grain Cd genotypes in bread wheat. Six parents were selected based on their Cd tolerance and were genotyped using triple-RAPD and ISSR markers to investigate their genetic diversity. The selected parents were crossed and the realized F_{1s} were selfed to produce F_2 populations and were backcrossed with their own parents to produce BC_1 and BC_2 populations. Six populations for each cross comprised P_1, P_2, F_1, F_2, BC_1 and BC_2 were evaluated in two adjacent experiments under non-Cd stressed and Cd-stressed conditions. Significant positive relative and standard heterosis were detected for flag leaf area, leaf chlorophyll content, proline content, Cd concentration and grain yield/plant under Cd-stressed condition. Dominance gene effect was more pronounced in controlling the evaluated traits in most cases. F values coupled with $F/\sqrt{H\times D}$ ratio were positive for Cd concentration and Cd sensitivity index in the three crosses under both conditions. Heritability estimates from offspring regression were high (< 50%) for flag leaf area, leaf chlorophyll content, proline content, Cd concentration while, moderately low for grain yield/plant and Cd sensitivity index. Prediction results revealed to high transgressive segregates and exceeding F_1 with best-inbred line (P max) that have all favorable alleles were obtained from 3rd cross for flag leaf area, low Cd concentration and Cd sensitivity index under Cd-stressed conditions.

Introduction

Heavy metals are one of the main obstacles that seriously threaten food safety (Konate *et al*., 2017; Rizvi *et al*., 2020). Cadmium (Cd) is one of the most prevalent toxic heavy metals that negatively impact plant growth and development (Tolcin, 2009; Shafiq *et al*., 2019). Cd is usually released from industrial activities as plastic manufacturing, refining and mining (Dong *et al*., 2019). Furthermore, increasing consumption of phosphate fertilizers and irrigation using wastewater leads to widespread Cd pollution in farmland (Zhang *et al*., 2015; Zaid *et al*., 2018). Cd causes numerous physiological disorders in lipid and protein synthesis, cell membrane and nutrient metabolism (Chugh and Sawhney 1999; Lesser, 2006). Moreover, it has destructive impacts on root growth and elongation (Atal *et al*., 1991; Black *et al*., 2014).

Wheat is the most important grain source for humans worldwide, and it is cultivated on more land areas than any other field crop (FAOSTAT, 2021). Wheat Cd contamination poses a substantial health risk (Nordberg, 2009). The European Food Safety Authority has lowered the tolerable weekly intake of Cd from 7 to 2.5 µg Cd^{-1} kg^{-1} body-weight (Adeniji *et al*., 2010; Singh *et al*., 2011). Long-term human exposure to Cd even at a low rate causes impaired kidney function, bone demineralization, emphysema, and proteinuria, and increases the threat of lung cancer (Bernard, 2008; Nordberg, 2009).

The hazard of Cd contamination highlights the importance of breeding for Cd-tolerant and low-grain Cd wheat genotype. Noticeable genotypic differences are detected in grain-Cd accumulation in bread wheat (Clarke *et al*., 1997; Stolt *et al*., 2003; Arduini *et al*., 2014; Guo *et al*., 2018). Developing tolerant wheat genotypes to Cd-stress with low Cd uptake into grains is the most efficient approach to reduce health threats as well as mitigate its negative impacts on plant growth (Zaid *et al*., 2018). Breeding Cd-tolerant wheat genotypes with lower Cd content requires crossing among superior individuals followed by selection in succeeding generations based on related traits to Cd-stress (Oladzad-Abbasabadi *et al*., 2018). Exploitation of heterosis provides an efficient perspective for enhancing wheat Cd potential and tolerance to Cd-stressed. Nevertheless, there remain several restrictions to breeding low-Cd wheat cultivars as slow and high cost of genetic improvement process as well as consuming long time. Developing low-Cd content and Cd-tolerant requires reliable understanding of natural genetic variation and inheritance of associated traits which is less characterized for hexaploid bread wheat. Therefore, the present investigation aimed at investigating the genetic diversity among bread wheat genotypes using triple RAPD and ISSR markers and assessing heterotic effects, genetic parameters, expected response from selection and prediction for low-Cd content and Cd tolerance.

Materials And Methods

2.1. Investigating the genetic diversity among selected parents

Twenty diverse bread wheat genotypes were screened for Cd tolerance in a preliminary experiment. The highly-tolerant six genotypes were selected to be crossed; namely Giza-168, Sids-6, ACSAD-925, Gemmeiza-10, ACSAD-935 and Line-1 (Table S1). The genetic diversity among selected parents was investigated using triple-RAPD and ISSR markers. DNA was extracted from young and fresh leaves (0.1 g) of the selected parents by the CTAB (cetyltrimethylammonium bromide). The quantity and quality of extracted DNA were measured (2 µl) by a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Delaware, USA). The DNA samples were altered to a concentration of 50 ng μl^{-1} with ddH_2O and used for PCR amplification.

Triple-RAPD-PCR reaction was applied as described by Williams et al. (1990) in volumes of 25 µl. The reaction mixture including 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, 100 µM of each dATP, dGTP, dCTP and dTTP (Pharmacia), 0.2 µl primer, 25 ng of genomic DNA, and 0.5 unit of Taq DNA polymerase (Promega). To increase the potential of PCR reaction, different combinations of three decamer oligonucleotides had been utilized in the single-primer PCR as suggested by Klein-Lankhorst et al. 1991 (Table 1).

Table 1. RAPD primers and ISSR primers applied for diversity screening (sequences 5'- 3')

RAPD	Sequences	ISSR	Sequences
P1	GTAGACCCG	814	(CT)8TG (#814)
P2	GGACCCTTAC	844A	(CT)8AC (#844A)
P3	GTCGCCGTCA	844B	(CT)8G (#844B)
P4	GGTCCCTGAC	17898A	(CA)6AC(#17898A)
P5	TGGACCGGTG	17898B	(CA)6GT (#17898B)
P6	AGGGGTCTTG	17899A	(CA)6AG (#17899A)
P7	TTCCCCCGCT	17899B	(CA)6GG (#17899B)
P8	TTCCCCCCAG	HB8	(GA)6GG (#HB8)
P9	ACTTCGCCAC	HB9	(GT)6GG (#HB9)
P10	CAATCGCCGT	HB10	(GA)6CC (#HB8)
P11	AGGGAACGAG	HB11	(GT)6CC (#HB11)
P12	TGCGCCCTTC	HB12	(CAC)3GC(#HB12)
P13	TTCGCACGGG	HB13	(GAG)3GC (#HB13)
P14	GTGAGGCGTC	HB14	(CTC)3GC (#HB14)
P15	CAAACGTCGG	HB15	(GTG)3GC (#HB14)
P16	CTGCTGGGAC		
P17	GTGACGTAGG		
P18	CCACAGCAGT		
P19	TGAGCGGACA		
P20	GTGAGGCGTC		

A set of fifteen ISSR primers was obtained from Metabion, Germany (Table 1). PCR amplification was applied as outlined by Dangi *et al.* (2004). Twenty ng of DNA was mixed with 50 mM KCl, 10 mM Tris-HCl pH 7.5, 0.5 mM spermidine, 1.5 mM MgCl2, 0.1 mM dNTPs, 0.8 U of Taq DNA polymerase and 0.3 uM primer in a 25 µl reaction utilizing Perkin Elmer 2400 thermocycler. All used chemicals for the reaction were procured from Sigma-Aldrich, USA.

Agarose gel electrophoresis (1.6%) was used for separating the amplified fragments. The fragments were recorded using EG-Gel Analyzer V1 software. The genetic similarity among parents was investigated by Nei's genetic distance (Nei, 1987). The dendrogram was performed using the Unweighted Pair Group Method with Arithmetic averages (UPGMA). The estimates were applied using the NTSYS-pc 2.02 software package (Numerical Taxonomy System, Exeter Software, Rohlf, 2000).

2.2.Crossing among selected parents

Three crosses were performed between the selected genetically diverse six parents; Giza-168×Sids-6 (1st cross), ACSAD-925×Gemmeiza-10 (2nd cross) and ACSAD-935×Line-1 (3rd cross). The F_1 populations were selfed to produce F_2 populations and were backcrossed with their own parents to produce BC_1 and BC_2 populations. Six populations for each cross; P_1, P_2, F_1, F_2, BC_1 and BC_2 were evaluated in two adjacent experiments in a randomized complete blocks design with three replications at the experimental farm of the Faculty of Agriculture, Zagazig University, Egypt (30°34'10" N 31°34'20" E). The first experiment was sprayed with cadmium solution at the beginning of heading stage by a concentration of 30 ppm Cd ion/liter of water (475 liter/ha). The second experiment was used as a control with pure water spraying. Seeds of six populations were sown in the fourth week of November. Rows were 2.5-m long and 20-cm apart, while a plant to plant space was 10-cm. The recommended agricultural practices for wheat production in the region were applied.

2.3. Measured traits

Data were recorded on individual guarded plants for the evaluated populations. Flag leaf area was determined at the time of full emergence of main spike. Flag leaf chlorophyll content was measured by SPAD-502 apparatus. Proline content in leaves was estimated as described by Bates *et al.* (1973) and grain yield/plant was assessed. For measuring Cd concentration, dried grain samples were weighed and followed by digestion at 160°C in 0.5 ml of concentrated glass-distilled HNO_3. A mixture of HNO_3: $HClO_4$ (0.25 ml) by 1:1 was mixed with the acid digestion residue and the digestion was continued at 200°C to dryness. The dry residue was dissolved in 1 ml of 8 n HNO_3, then diluted 10:1 with d1 H_2O and analyzed for Cd via inductively coupled argon plasma emission spectrometry (Model ICAP 61E; Thermo-Jarrell Ash, Waltham, MA, USA), (Hart *et al.*, 2005).

2.4. Statistical analysis and biometrical assessments

Analysis of variance for all evaluated traits was done using SAS Software. An index of Cd sensitivity (CSI) was computed as described by Fisher and Maurer (1978) using the following equation: Cadmium Sensitivity Index $(\mathrm{CdSI}) = \frac{\left[1-\left(\frac{Ys}{Yp}\right)\right]}{SI}$ where, Ys and Yp are the grain yield/plant of each genotype under

stress and normal conditions, respectively. SI is stress intensity = $1-\left(\frac{\bar{y}s}{\bar{y}p}\right)$, $\bar{y}s$ and $\bar{y}p$ are averages of grain yield for all genotypes under Cd-stressed and non-stressed conditions, in the same order. Mid-parents heterosis and standard heterosis were calculated using the formula outlined by Bitzer *et al.* (1982) as follows: Mid-parents heterosis $(\mathrm{MPH}_{\%}) = \frac{[F1-MP]}{MP} \times 100$. Standard heterosis $(\mathrm{SH}_{\%}) = \frac{[F1-\text{Check cultivar}]}{\text{Check cultivar}} \times 100$. Inbreeding depression = $\frac{[F1-F2]}{F2} \times 100$, where, MP is mean of mid parents, and check cultivar is average of Giza-168. F_2 deviation was calculated according to Sun et al. (1972) as follows: F_2 deviation = $F_2 - 0.5[F_1 + 0.5(P_1 + P_2)]$. Parent-offspring regression (h^2), realized heritability (RH), and genetic advance from selection ($Gs_{\%}$) were also computed according to Falconer (1989). The components of the genetic variance i.e. additive VD, dominance VH and environmental VE variances were estimated as described by Mather and Jinks (1982) and were utilized further to calculate frequency between dominance and recessive alleles in the parental populations F = (VBC_2+ VBC_1) and the dominance at different loci (F/√ H × D).

2.5. Predicting properties of new recombinant lines

The properties of new recombinant lines that fall outside the parental range and exceeding F_1 hybrid following selfing generations were calculated using Jinks and Pooni (1976) formula. The proportion of inbreds falling outside parental range = d/√D, the proportion of inbreds exceeding F_1 hybrid = h/√D. Also, the best inbred (P max) = m + h/√H×D was calculated according to Hayward *et al.* (1993) Where: m = 0.5(P_1 + P_2), [d] = 0.5 (P_1-P_2) and [h] = F_1–m, where D is additive genetic variance and H is dominance genetic variance.

Results And Discussion

3.1. Genetic diversity among selected parents

Utilizing molecular markers increases the efficacy of classical plant breeding by assessing the genetic diversity among used parents (Milad *et al.*, 2011; Pozniak *et al.*, 2012; Abu Hammad *et al.*, 2016). In the current study, genetic diversity was investigated using triple-primer RAPD and ISSR markers. Triple-RAPD amplification reactions were applied using different combinations of three different decamer oligonucleotides that had been previously tested in the single-primer PCR (Table 1). In all cases, the combination of three primers (in 1: 1: 1 ratio) led to appearance of new bands that were not amplified using each primer independently (Fig. 1A). The sizes of produced fragments varied from 100 bp to 2 Kbp. A total of 440 bands were recorded, in average 35 ± 2% band per primer/gel, 12 ± 2 % polymorphic, 25 ± 2% unique bands and 36 ± 2% polymorphic (with unique), which revealed 70 to 80% polymorphism. While certain bands were monomorphic in all genotypes, there were specific bands for each one (Fig. 1A). Genetic similarity was determined by Nei's index value for all genotypes considering Triple-RAPD results, then were employed to perform dendrogram using Unweighted Pair Group Method with Arithmetic averages (UPGMA) (Fig. 1B, C). The dendrogram displayed genetic diversity among used wheat parents.

ISSR technique utilizes frequently 16-25 bp long primers in a single primer PCR reaction focusing on multiple genomic loci to amplify principally the inter-SSR sequences of different sizes (Ziêtkiewicz *et al.*, 1994). In the current study, a set of 50 ISSR primers was applied for preliminary screening of six wheat genotypes (Fig. 1A). However, only fifteen ISSR primers identified intraspecific variation in wheat genotypes produced on average fifteen bands per gel/primer in the range of 100 bp to 2 kbp. Among these bands, four were polymorphic bands and sixteen were unique bands revealing polymorphism. Based on ISSR gels patterns, the similarity index values were employed to create dendrogram utilizing UPGMA. The obtained dendrogram showed different clusters displaying variation in the frequencies of SSR motifs (Fig. 1B, C). Likewise, Pozniak *et al.* (2012); Abu Hammad *et al.* (2016) and Liu *et al.* (2019) demonstrated the potentiality of molecular markers to distinguish the parental diversity based on Cd tolerance.

3.2. Heterosis and F_2 deviation

The reliability of genetic components depends mainly on the amount of genetic variability among the used parents. The obtained results indicated positive and significant relative and standard heterosis for flag leaf area in the three investigated crosses under both conditions. Likewise, leaf chlorophyll content in the 2nd cross exhibited positive and significant relative and standard heterosis under both conditions as well as the 1st cross under Cd-stressed while 3rd cross for standard heterosis only under Cd-stressed (Table 2). Besides, significantly positive relative heterotic effects were observed for proline content in 1st and 3rd crosses under non-Cd stress and 2nd cross only under Cd-stressed conditions. Desirable heterosis over mid-parents or standard cultivar has been registered for grain yield/plant in the 1st and 2nd crosses under both conditions and the 3rd cross only under non-Cd stressed conditions. On the other hand, negative heterosis in the desired direction was detected for Cd concentration in the 2nd cross under non-Cd stressed and standard heterosis in 1st and 2nd crosses under Cd-stressed as well as for Cd sensitivity index in 1st and 2nd crosses for mid-parent heterosis. These crosses accumulated lower amounts of Cd concentration rather than the other crosses *i.e.* 3rd cross under both conditions which accumulated higher amounts of cadmium in grains. Cd sensitivity index displayed significant positive relative heterosis in the 3rd cross. The obtained significant relative and standard heterosis were due to heterotic effects and dominance and/or dominance×dominance gene effects in the evaluated crosses. Likewise, significant heterotic effects for agronomic traits and Cd concentration were recorded by Clarke *et al.* (1997). Similarly, Awaad *et al.* (2013) recorded significant heterobeltiosis for flag leaf area, leaf chlorophyll content, proline content, Cd concentration and grain yield/plant under both non-Cd stressed and Cd-stressed conditions.

Table 2
Estimates of heterosis, inbreeding depression, dominance and F_2 deviations for evaluated characters of the three wheat crosses under non-Cd-stressed and Cd-stressed conditions.

Parameter	Flag leaf area (cm^2)			Leaf chlorophyll content (SPAD value)			Proline content (µ moles /g FW)		
	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st Cross	2nd cross	3rd cross
	Non-Cd-stressed conditions								
Mid-parents heterosis	12.74**	10.13**	16.71**	0.72	4.65**	-4.88**	44.57**	-4.62*	11.44**
Standard heterosis	7.13*	31.22**	33.31	2.95	6.45**	-1.09	13.68*	-0.31	-15.46*
Inbreeding depression	2.72	19.02**	5.39*	-4.91**	3.03**	1.25	-29.32**	9.69*	16.79**
Dominance deviation	5.40*	4.56*	7.70**	0.35	2.20*	-2.71*	0.41**	-0.16	0.28*
F_2 deviation	1.40*	-7.15**	0.95	2.58*	-0.40	-2.03*	0.59**	-0.39*	-0.31*
	Cd-stressed conditions								
Mid-parents heterosis	24.37**	10.33*	19.41**	21.48**	4.24*	0.51	11.35**	-1.53	-5.53**
Standard heterosis	18.04**	31.17**	44.11**	27.59**	6.24*	4.78*	-0.96	6.94*	-16.07*
Inbreeding depression	6.43*	19.77**	6.63*	-0.59	2.03**	-1.79*	7.77*	35.34*	15.43**
Dominance deviation	8.90**	3.98*	8.09**	7.85**	1.80*	0.26	0.21**	-0.08	-0.22*
F_2 deviation	1.53*	-6.41**	0.75	3.67**	0.00	1.03*	-0.06	-1.84*	-0.69*
	Cd concentration (mg Cd/kg DW)			Grain yield per plant (g)			Cd sensitivity index		
	Non-Cd-stressed conditions								
Mid-parents heterosis	11.42**	-4.08*	15.19**	27.12**	26.33**	15.96**	-23.74*	-9.83*	96.72**
Standard heterosis	2.04*	-16.96**	51.23**	10.21*	40.16**	-15.41*	-32.59*	-41.05**	-57.87**
Inbreeding depression	-18.50**	2.13	17.59**	12.24**	0.56	10.44*	48.74**	7.98*	28.23**
Dominance deviation	0.021*	-0.01	0.04*	1.94*	1.86*	1.35*	-0.48**	-0.15	0.48
F_2 deviation	0.05**	-0.01	-0.03*	-0.14	0.88*	-0.35	-0.99**	-0.18*	-0.04
	Cd-stressed conditions								
Mid-parents heterosis	0.53	3.52*	9.39*	44.69**	28.21**	-2.85*			
Standard heterosis	-7.04**	-13.04*	15.75*	33.40**	59.69**	-30.66**			
Inbreeding depression	-3.03*	16.67**	13.07**	-3.15*	-1.24*	1.26*			
Dominance deviation	0.01	0.03*	0.08*	2.06**	1.59*	-0.21			
F_2 deviation	0.02**	-0.12*	-0.08*	1.24**	0.89*	-0.19			

Inbreeding depression and dominance deviations displayed a similar trend and were found to be significantly positive for flag leaf area in the three crosses, leaf chlorophyll content in the 2nd cross and Cd concentration in the 3rd cross under both conditions. Similarly, inbreeding depression and dominance deviations were significantly positive for proline content in 1st cross and Cd concentration in 2nd cross under Cd-stressed as well as grain yield/plant in 1st and 3rd crosses under non-Cd stressed conditions. These results could be discussed on the basis of heterotic effects and dominance and/or dominance × dominance gene effects in the assessed crosses. On the contrary, decreasing alleles were involved in the inheritance of Cd concentration in 1st cross under both conditions and grain yield/plant in 1st and 2nd crosses under Cd-stressed conditions. Otherwise, dominance deviation exhibited significantly positive values for grain yield/plant in 2nd cross under non-Cd stressed condition and 1st and 2nd crosses under Cd-stressed. Moreover, dominance deviation exhibited negative and significant values for leaf chlorophyll content in 3rd cross under non-Cd stressed and proline content under Cd-stressed as well as Cd

sensitivity index in the 1st cross (Table 2). F_2 deviation exhibited significant positive estimates for flag leaf area, leaf chlorophyll content and Cd concentration in 1st cross; grain yield/plant in 2nd cross under both conditions; leaf chlorophyll content in 3rd cross under Cd-stressed. Otherwise, it was negative and significant for flag leaf area, Cd concentration in 3rd cross; proline content in 2nd and 3rd crosses under both conditions; leaf chlorophyll content in 3rd cross under non-Cd stressed as well as Cd sensitivity index in 1st and 2nd crosses.

3.3. Gene effect and heritability

The nature of gene action and heritability play an important role in identifying the appropriate breeding method to improve economic traits through breeding programs. Genetic parameters controlling cadmium stress tolerance and related traits are presented in Table (3). The additive gene effect [d] was significant and involved in the genetics of flag leaf area in 1st cross and proline content in 3rd cross under non-Cd stress as well as in 1st cross under Cd-stress conditions. Likewise, significant additive gene effect was detected for Cd concentration in 1st cross under both conditions and 2nd and 3rd crosses under non-Cd stressed conditions. Hereby, reflecting in an h/d ratio was less than unity, showing no over dominance. Liu et al. (2019) revealed the significance of additive effect with high heritability for grain Cd concentration suggests the opportunity of breeding consistently low-Cd wheat cultivars crossways environments.

The dominance gene effect [h] which indicate the presence of heterotic effects and dominance and/or dominance×dominance gene effects was significant and involved in controlling flag leaf area, leaf chlorophyll content in the three crosses; grain yield/plant in 1st and 2nd crosses under both conditions; proline content in 1st and 2nd crosses under non-Cd stressed and 2nd and 3rd under Cd-stressed; Cd concentration in 1st cross under non-Cd stressed and 2nd and 3rd crosses under Cd-stressed and Cd sensitivity index in 1st and 3rd crosses, reflecting potency ratio h/d was more than unity (Tables 3 and 4). These results indicated the presence of dominant genes which increase expression between the parents and ensure transgressive segregation for these traits in the F_2 generation. Otherwise, h/d ratio was less than unity for proline content in 3rd cross and Cd concentration in 2nd and 3rd crosses under non-Cd stressed condition as well as for proline content in 1st cross and Cd concentration in the three crosses under Cd-stressed indicating partial dominance occurred. The additive and over-dominance type of genetic architecture are previously detected for flag leaf area; leaf chlorophyll content, Cd content, proline content and grain yield/plant by Awaad et al. (2013). Furthermore, EL-Gharbawy et al. (2015) disclosed that both additive and dominance gene effects were involved in controlling Cd and proline contents with a greater role for dominance and relatively high narrow-sense heritability in respect to proline content. On the other hand, Dunwei *et al.* (2012) manifested that Cd tolerance in wheat was governed by additive genetic variance.

Table 3
Measured genetics parameters controlling Cd-stress tolerance in the evaluated traits of three wheat crosses under non-Cd-stressed and Cd-stressed conditions.

Parameter	Flag leaf area (cm^2)			Leaf chlorophyll content (SPAD value)			Proline content (µ moles /g FW)			Cd concentration (mg Cd/kg DW.)			Grain yield per plant (g)		
	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross
	Non-Cd-stressed conditions														
m	42.39	45.04	46.09	48.55	47.3	56.41	0.92	3.36	2.41	0.18	0.25	0.27	7.14	7.05	8.43
d	2.22*	-7.24*	-5.74*	-1.05*	-0.80	-2.12*	0.25	-0.15	0.77*	0.02	0.04*	-0.06*	1.09	-0.69	3.13*
h	-8.20**	9.74**	-9.60**	-5.50*	-1.40*	6.76*	-1.60**	0.93*	0.35*	-0.12*	0.03*	0.03*	-1.65*	-3.61*	-0.65*
h/d	-3.69	-1.35	1.67	5.24	1.75	-3.19	-6.40	-6.41	0.46	-6.97	0.79	-0.43	-1.51	5.19	0.21
F	1.53	-8.06	-9.36	9.51	8.52	0.76-	-0.01	0.13-	-0.57	0.001	0.03	0.004	0.16	-0.09	-0.48
F/√ H × D	0.29	-0.20	-0.24	0.19	0.16	-0.22	-0.04	-0.31	-1.27	0.06	0.60	0.29	0.06	-0.06	-0.13
Heritability															
h^2	0.70	0.67	0.53	0.80	0.62	0.54	0.82	0.70	0.76	0.84	0.88	0.90	0.31	0.44	0.45
RH	0.55	0.79	0.65	0.93	0.89	0.58	0.98	0.85	0.85	0.78	0.86	0.89	0.27	0.26	0.45
Gs%	6.54	23.81	13.19	23.41	26.22	3.99	77.59	37.65	60.64	79.15	82.47	81.43	10.71	9.02	16.89
	Cd-stressed conditions														
m	36.52	38.52	41.70	36.55	42.50	50.15	1.85	5.18	3.98**	0.66	0.75	0.85	4.61	5.66	7.38
d	1.96	-6.10*	-7.15**	-1.75	-0.80	2.05*	0.23*	-0.41*	-0.50	0.05*	0.14*	-0.05*	0.39	-1.12	2.98*
h	-11.96**	8.84**	-9.59**	-15.18**	-1.80*	2.31*	-0.10	3.76**	1.60*	-0.05	0.21*	0.08*	-4.54**	-3.37**	0.60*
h/d	-6.10	-1.45	1.34	8.67	2.25	1.13	-0.44	-9.17	-3.20	-0.88	1.44	-1.79	-11.64	3.02	0.20
F	15.09	-19.09	-6.49	-6.42	18.70	-0.32	-0.47	0.66	0.01	0.001	0.001	0.01	-0.51	0.27	0.16
F/√ H × D	0.51	-0.85	-0.08	-0.05	0.22	-0.32	-0.55	3.13	0.02	0.02	0.05	0.19	-0.19	0.23	0.04
Heritability															
h^2	0.60	0.55	0.40	0.60	0.58	0.45	0.60	0.55	0.64	0.7	0.5	0.84	0.26	0.30	0.27
RH	0.37	0.69	0.52	0.76	0.72	0.44	0.87	0.80	0.85	0.79	0.35	0.34	0.19	0.11	0.26
Gs%	10.39	16.63	14.69	27.68	26.00	3.02	52.05	24.54	29.36	39.53	19.81	10.06	8.23	5.59	11.99

h^2 is heritability estimates from parent-offspring regression, RH is realized heritability and Gs% is genetic advance from selection

Table 4
Estimated genetic parameters for Cd sensitivity index of three wheat crosses under non-Cd-stressed and Cd-stressed conditions.

Parameter	3rd cross	2nd cross	1st cross
m	2.03	1.50	0.49
d	0.27	0.59	-0.11
h	1.24**	0.51*	-0.41**
h/d	4.64	0.87	3.63
F	0.16	0.70	0.10
F/√ H × D	0.11	0.45	0.08
Heritability			
h^2	0.28	0.45	0.32
RH	0.20	0.24	0.20
Gs %	76.11	79.19	83.55

Table 5
Predicted properties of recombinant lines exceeding parental range for evaluated traits of three wheat crosses under non-Cd stressed and Cd-stressed conditions.

Parameter	Flag leaf area (cm^2)			Leaf chlorophyll content (SPAD value)			Proline content (µ moles /g. FW)		
	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross
	Non-Cd-stressed conditions								
m = F2	46.50 ± 1.85	40.17 ± 1.75	50.90 ± 2.23	51.30 ± 1.45	48.00 ± 3.09	53.03 ± 1.18	1.720 ± 0.33	2.890 ± 0.45	2.230 ± 0.43
Range of inbreds m ± 2√D	41.57–51.43	23.95–56.39	36.50–65.29	34.24–68.36	24.93–71.07	48.58–57.48	0.13–3.57	1.12–4.66	0.21–4.25
Probability (d/√D)	0.90	0.89	0.79	0.12	0.07	0.95	0.27	0.164	0.757
Proportion of inbreds *	18.41	18.67	21.48	45.22	47.21	17.11	39.36	43.64	22.36
	Cd-stressed conditions								
m = F2	42.50 ± 1.45	34.10 ± 1.90	46.50 ± 2.07	44.14 ± 1.42	43.40 ± 1.15	51.30 ± 1.00	1.900 ± 0.19	3.300 ± 0.42	3.18 ± 0.224
Range of inbreds m ± 2√D	34.24–50.76	22.39–45.82	27.09–65.91	20.99–67.28	21.29–65.51	47.39–55.21	0.03–3.77	1.63–4.97	1.52–4.84
Probability (d/√D)	0.48	1.04	0.74	0.15	0.07	1.05	0.25	0.49	0.60
Proportion of inbreds *	31.56	14.92	22.97	44.04	47.21	14.69	40.13	31.21	24.51
	Cd concentration (mg Cd/kg DW.)			Grain yield per plant (g)			Cadmium sensitivity index		
	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross
	Non-Cd-stressed conditions								
m = F2	0.24 ± 0.041	0.23 ± 0.05	0.25 ± 0.05	7.96 ± 0.43	8.85 ± 0.42	8.75 ± 0.64	0.79	1.25	0.69
Range of inbreds m ± 2√D	0.02–0.49	0.02–0.48	0.69–1.19	4.84–11.08	6.79–10.91	5.02–12.48	1.66–3.24	1.25–3.74	1.37–2.75
Probability (d/√D)	0.13	0.31	0.134	0.701	0.674	1.674	0.267	0.586	-0.115
Proportion of inbreds *	44.83	37.83	44.83	24.19	25.14	4.75	41.29	31.92	45.62
	Cd-stressed conditions								
m = F2	0.68 ± 0.06	0.650 ± 0.124	0.81 ± 0.07	6.88 ± 0.40	7.34 ± 0.56	7.08 ± 0.45			
Range of inbreds m ± 2√D	0.24–1.12	0.36–0.94	0.69–0.91	4.41–9.35	5.78–8.89	3.51–10.65			
Probability (d/√D)	0.25	0.98	0.85	0.32	1.43	1.67			
Proportion of inbreds *	40.52	16.35	19.77	37.45	7.64	4.85			

* Proportion of inbreds falling outside parental range

F values indicate the frequency between dominance and recessive alleles in the parental populations. Also, F/√(H×D) provided evidence that the dominance at different loci particularly consistent in sign or magnitude. F value and F/√(H×D) ratio were positive for flag leaf area in 1st cross; leaf chlorophyll content in 2nd cross; Cd concentration as well as Cd sensitivity index in the three crosses under both environments and leaf chlorophyll content and grain yield/plant in 1st cross under non-Cd stressed conditions. The positive F value indicates that dominant alleles were more frequent than recessive ones in the parental populations. Whereas, both parameters were negative for flag leaf area in 2nd and 3rd crosses; leaf chlorophyll content in 3rd cross; proline content in 1st cross under both conditions; proline content and grain yield/plant in 2nd and 3rd crosses under non-Cd stressed conditions. Negative F values revealed that recessive alleles were more frequent than dominant ones in the parental populations.

Heritability computed from parent-offspring regression (h^2) and realized heritability (RH) are shown in Tables (3) and (4). Estimates from parent-offspring regression (h^2) were high (<50%) for flag leaf area, leaf chlorophyll content, proline content and Cd concentration in most studied crosses under both conditions. While, it was moderate for flag leaf area and leaf chlorophyll content under Cd-stressed, also varied from low to moderate for grain yield/plant and Cd sensitivity index. Realized heritability (RH) recorded values less than h^2. Generally, RH was high for flag leaf area, leaf chlorophyll content, proline content and Cd concentration under non-Cd stressed conditions. However, it varied from moderate (37.4%) to high (87.0%) for that traits under Cd-stress as well as low for grain yield/plant and Cd sensitivity index under both environments. Also, low to moderate heritability estimates were registered in the remaining crosses for the different traits under both conditions. Genetic advance as percentage of the population mean was high for proline content, Cd concentration under non-Cd stress and moderate under Cd-stress and detected to be high for Cd sensitivity index, and varied from low to moderate for the remaining traits, under both conditions. The simple inheritance based on high heritability and genetic advance observed herein indicates substantial progress could be achieved through

selection for low Cd concentration (Clarke *et al*., 2002;, Ishikawa *et al*. 2010; Oladzad-Abbasabadi *et al*. 2018 and Liu *et al*. 2019). Similar results were recorded for morpho-physiological traits, Cd concentration and grain yield/plant by Clarke *et al*. (1997); Awaad (2003) and Awaad *et al*. (2010).

3.4. Predicting properties of new recombinant lines

Predicted properties of new recombinant lines that fall outside the parental range and exceeding F1 hybrid are presented in Tables (5) and (6). The range of inbreds m ± 2√D was lower for flag leaf area, leaf chlorophyll content and grain yield/plant under Cd-stress compared with non-Cd stress conditions as a result of Cd effect on gene action. On the contrary, m ± 2√D under Cd-stress was lower for proline content and Cd concentration rather than non-Cd stress, reinforcing the possibility to isolate great number of lines more tolerant to Cd pollution after selfing generations. The results exhibited expected transgressive segregates that outperform the parental range. The highest percentages of such segregants under non-Cd stressed conditions were recorded by 3rd cross for flag leaf area (21.48%) and Cd concentration (44.83%) while 2nd cross for leaf chlorophyll content (47.21%), proline content (43.64%) and grain yield/plant (25.14%). Similarly, 3rd cross displayed highest percentages of segregants for cadmium sensitivity index (45.62%). On the other hand, under Cd-stressed conditions, the highest percentage of recombinant lines exceeding parental range was recorded by 1st cross for flag leaf area was (31.56%), proline content (40.13%), Cd concentration (40.52%) and grain yield/plant (37.45%). While 2nd cross for leaf chlorophyll content (47.21%). The recombinants that showed stability from non-Cd stressed to Cd-stressed conditions were assigned for 2nd cross in leaf chlorophyll content and 3rd cross for grain yield/plant, but fluctuated from non-Cd stressed to Cd-stressed in the other crosses.

The highest proportion of inbreds exceeding F1 under non-Cd stressed was recorded by 2nd cross for flag leaf area (11.51%), leaf chlorophyll content (45.22%), while by 3rd cross for proline content (36.32%), Cd concentration (47.61%), grain yield/plant (36.32%) and Cd sensitivity index (34.83%) (Table 6). Moreover, under Cd-stress the highest proportions exceeding F1 was recorded by 3rd cross for flag leaf area was (48.80%) and grain yield/plant (36.69%), while 2nd cross for leaf chlorophyll content (43.64%) and 1st cross for proline content (45.62%) and Cd concentration (41.29%). The best-inbred line (P max) that will have all favorable alleles tended to decrease from non-Cd stressed to Cd-stressed as a result of Cd effect (Table 6). P max was recorded by 3rd cross for flag leaf area, Cd concentration and grain yield/plant, while 2nd cross for leaf chlorophyll content and proline content under non-Cd stressed. Whereas under Cd-stressed, the best-inbred line (P max) was registered by 2nd cross for flag leaf area, leaf chlorophyll content, proline content and Cd concentration while 3rd cross for grain yield/plant. Similar interpretation was stated by Mather and Jinks (1982) and also Awaad (2002) elucidated high proportion of recombinants falling outside parental range and exceeding F1 for grain yield/plant and morpho-physiological traits.

Table 6. Predicted properties of recombinant lines exceeding F_1 for evaluated traits of the three wheat crosses under non-Cd-stressed and Cd-stressed conditions.

Parameters	Flag leaf area (cm^2)			Leaf chlorophyll content (SPAD value)			Proline content (µ moles /g. FW)			Cd concentration (mg Cd/kg DW)			Grain yield per plant (g.)			Cad inde
	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	1st cros
	Non-Cd-stressed conditions															
Probability (h/√D)	3.33	1.20	1.33	0.65	0.12	3.04	1.73	1.05	0.35	0.91	0.25	0.06	1.06	3.50	0.35	1.01
Proportion of inbreds*	0.05	11.51	9.18	25.79	45.22	0.12	4.18	14.69	36.32	18.14	40.13	47.61	14.46	0.23	36.32	15.6
P Max	54.87	66.44	71.22	73.72	120.29	78.10	4.82	6.14	6.06	0.33	0.44	1.48	7.41	6.26	9.31	1.45
	Cd-stressed conditions															
Probability (h/√D)	2.90	1.51	0.03	1.31	0.16	1.18	0.11	4.49	1.93	0.22	1.42	1.52	3.67	4.33	0.34	
Proportion of inbreds*	0.19	6.55	48.80	9.51	43.64	11.90	45.62	0.00	2.68	41.29	7.78	6.43	0.01	0.00	36.69	
P Max	26.39	64.96	55.97	46.45	64.94	46.49	2.12	16.88	3.97	0.71	1.22	0.53	3.96	3.75	5.90	

* Proportion of inbreds exceeding F1

Declarations

Author contribution: H.A.A, A.M.A., A.M.M., E.S.A.M. and E.M. conceived and designed the study; H.A.A, A.M.A., A.M.M., E.S.A.M. and E.M. performed the experiments; H.A.A, A.M.A. and E.M. analyzed the data; H.A.A, A.M.A. and E.M. interpreted the data of the experiments; H.A.A, A.M.A. and E.M. drafted the manuscript; H.A.A, A.M.A. and E.M. edited and revised manuscript, all authors approved the final version of the manuscript.

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Consent for publication Not applicable

Competing interests The authors declare no competing interests

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Figures

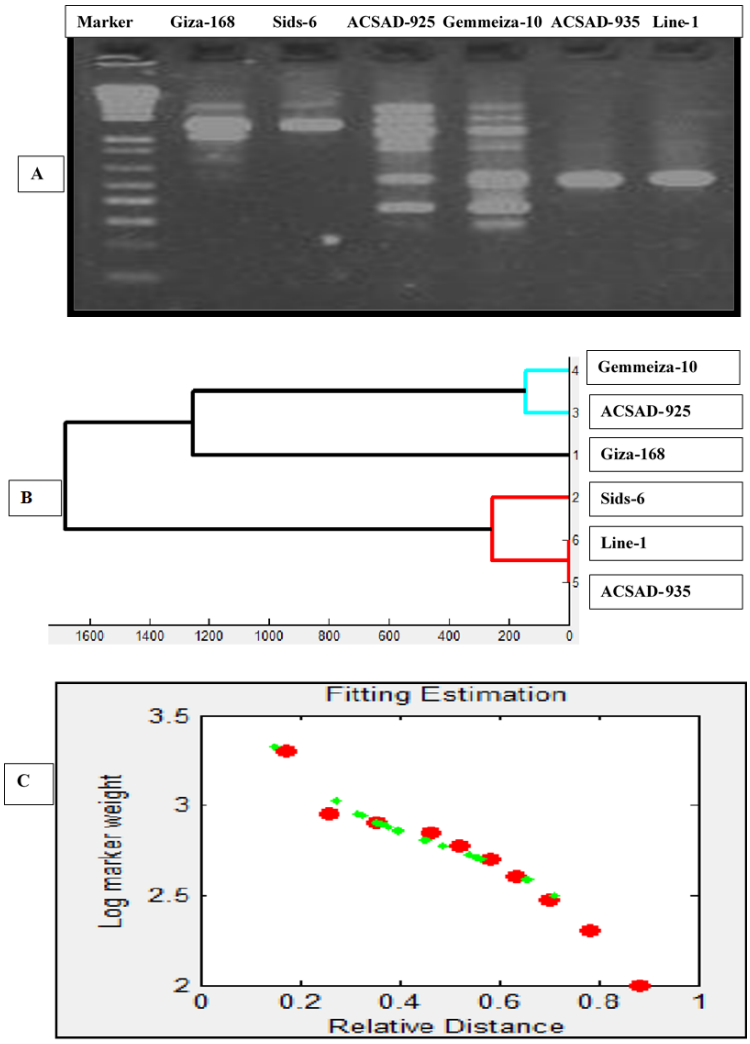

Figure 1

RAPD marker associated with polymorphisms among six wheat cultivars. A) RAPD amplification with triple-primers P3, P6 and P8. B) Dendrogram based on the algorithm of unweighted pair group method with arithmetic averages using RAPD results between in different wheat cultivars. C) Testing the accuracy of Triple-RAPD dendrogram using the fitting method plotting.

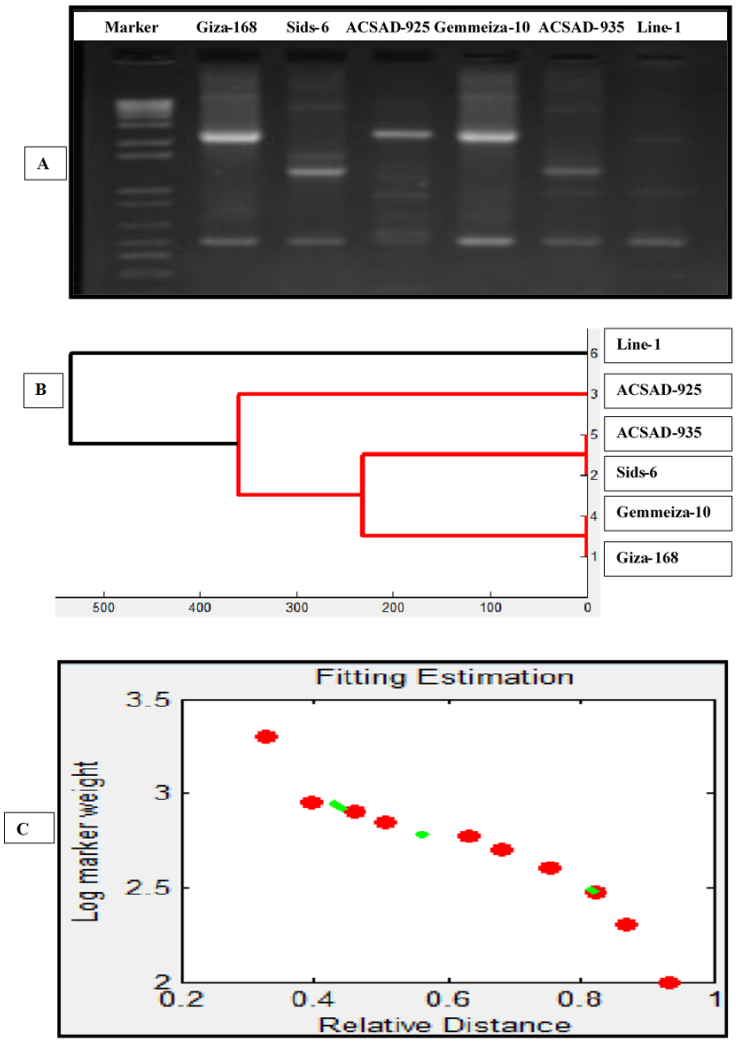

Figure 2

ISSR marker associated with polymorphisms among six wheat cultivars. A) PCR amplification with HB11 primer. B) Dendrogram based on the algorithm of unweighted pair group method with arithmetic averages using ISSR results between in different wheat cultivars in Egypt. C) Testing the accuracy of ISSR dendrogram using the fitting method plotting.

Supplementary Files

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- TableS1.docx