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RESEARCH ARTICLE

Genetic analysis of cadmium tolerance and exploration of its inheritance nature in bread wheat (*Triticum aestivum* L.)

Hassan A. Awaad, Ahmed M. Alzohairy[1], Amgad M. Morsy[2], Ehab S.A. Moustafa[3] and Elsayed Mansour*

Abstract

Cadmium (Cd) is a non-essential and extremely toxic element that destructively impacts agricultural production. Accordingly, developing Cd-tolerant genotypes with low-grain Cd is a promising approach to cope with the pollution problem. The current study aimed to understand the inheritance nature of Cd tolerance and the detection of Cd-tolerant bread wheat genotypes with low-grain Cd. 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, the realized F1s were selfed to produce F_2 populations, and F_{1s} were backcrossed with their own parents to produce B_1 and B_2 populations. Six populations for each cross comprised P_1, P_2, F_1, F_2, B_1 and B_2 were evaluated in two adjacent experiments under non-Cd stress and Cd-stress conditions. Significant positive relative and standard heterosis were detected for flag leaf area, leaf chlorophyll content, proline content, Cd content and grain yield/plant under Cd-stress conditions. Desirable heterosis was assigned for Cd content, proline content and yield traits in the 1st and 2nd crosses under Cd-stress conditions. These crosses produced higher grain yield while accumulating lower amounts of Cd in grains under Cd-stress conditions. Furthermore, prediction results revealed high transgressive segregates and exceeding F_1 with best-inbred lines with favorable alleles obtained from 1st and 2nd crosses for high-yielding and low Cd content under Cd-stress conditions. Both additive and dominance gene effects were involved in controlling Cd content, proline content,and yield traits.

Keywords: Cadmium, RAPD, ISSR, heterosis, heritability, prediction, sensitivity index

Introduction

Wheat is the most important grain source for humans worldwide, and it is cultivated on more land areas than any other field crops (FAOSTAT 2022). Its total cultivated area is around 219 million hectares, producing 761 million tons (FAOSTAT 2022). Egypt is involved in these statistics, with 1.3 million hectares and 8.8 million tons in production. Egypt is one of the top wheat importers, with approximately 10 million tons annually imported (FAOSTAT 2022). Besides, current and expected future population growth and climate change increase the gap between production and consumption (Moustafa et al. 2021; Swailam et al. 2021; Megahed et al. 2022).This calls for increasing its productivity and expanding the cultivated areas to marginal environments (Mansour et al. 2020; Desoky et al. 2021; Kamara et al. 2022).

Abiotic stresses are one of the major factors affecting crop growth and productivity of crops ascross the world. Plants are continuously confronting the harsh environmental conditions, such as soil salinity, alkalinity/sodicity, drought, cold, heat, flooding and heavy metal contamination (Gill and Tuteja 2011; Tao et al. 2015; Joshi et al. 2021, 2022; Singh et al. 2014). Heavy metals are one of the main obstacles that seriously threaten food safety (Rizvi et al. 2020). Cadmium (Cd) is one of the most prevalent toxic heavy metals among 20 toxin that negatively impact plant growth and development (Shafiq et al. 2019; Elgharbawy et al. 2021). Cd is usually released from industrial activities as plastic

Department of Crop Science, [1]Genetics Department, Faculty of Agriculture, Zagazig University, Egypt

[2]Wheat Research Department, Agricultural Research Center, Giza, Egypt

[3]Genetic Resources Department, Desert Research Center, Cairo, Egypt.

***Corresponding Author:** Elsayed Mansour, Department of Crop Science, Faculty of Agriculture, Zagazig University, Egypt, E-Mail: sayed_mansour_84@yahoo.es

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manufacturing, refining and mining (Dong et al. 2019; Yaciuk et al. 2022). It is rapidly taken up by roots and accumulated in different plant tissues, restricting crop growth and productivity (Halim et al. 2021). Furthermore, increasing consumption of phosphate fertilizers and irrigation using wastewater leads to widespread Cd pollution in farmland (Zaid et al. 2018).

Cd causes numerous biochemical and physiological disorders in lipid and protein synthesis, cell membrane stability index, nutrient metabolism rates of transpiration, and net photosynthesis (Zhao et al. 2021; Sardar et al. 2022). Besides, high levels of Cd exhibit a considerable reduction in photosynthetic pigments, leaf number and leaf area in wheat plants. The osmolyte contents are substantially increased as a result of the increased electrolyte leakage and malondialdehyde content and under Cd stress. Moreover, it has destructive impacts on root epidermis, growth, and elongation (Black et al. 2014; Qin et al. 2022). These adverse impacts of Cd stress reflected a considerable reduction in the number of grains per spike, 1000-grain weight, and final wheat productivity.

Contamination of wheat with Cd 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} bodyweight (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 (Nordberg 2009; Cirovic et al. 2022). The agricultural drainage water and domestic waste which suffer from intensive pollution, are frequently used in several regions for irrigation (Abdelrazek 2019). Moreover, phosphate fertilizers is regularly applied during soil preparation for crop growing. These factors contribute to the accumulation of Cd in many regions (Badawy et al. 2021). The hazard of Cd contamination highlights the importance of breeding for Cd tolerant genotypes with low-grain Cd. Developing tolerant genotypes to Cd stress with low Cd uptake into grains is a promising, eco-friendly and efficient approach to minimizing human dietary intake of Cd as well as mitigating its negative impact on plant growth (Zaid et al. 2018; Kamara et al. 2022). Although the low Cd genotypes should possess acceptable yield and quality with Cd concentration below maximum permissible limit for safe consumption (Chen and Wu 2020) however, noticeable genotypic differences in Cd accumulation in wheat grain have been detected (Arduini et al. 2014; Guo et al. 2018). The exploitation of heterosis provides an efficient perspective to enhance the potential for improving tolerance to Cd-stress in wheat.

However, there remain several restrictions to breeding low-Cd wheat cultivars as slow, time-consuming and high cost of the genetic improvement process. Moreover, breeding for Cd-tolerance requires a 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 studying 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

Investigating genetic diversity among parents

Twenty diverse bread wheat genotypes were screened for Cd tolerance in a preliminary experiment (data not shown). 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 1). 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 14-day-old seedlings of the selected parents by the CTAB (cetyltrimethyl ammonium bromide) method. The quantity and quality of extracted DNA were measured (2 μL) by a NanoDrop ND-1000 UV-Vis spectrophotometer (Nano Drop Technologies, Delaware, USA). The DNA samples were altered to a concentration of 50 ng μL^{-1} with ddH_2O and used forPCR amplification.

Triple-RAPD-PCR reaction was applied as Williams et al. (1990) described in volumes of 25 μL reaction mixture. The reaction mixture included 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

Table 1. Names, pedigree and origin of used wheat genotypes as parents

Name	Pedigree	*Origin*
Giza-168	MIL/BUC//Seri: CM 93046-8M-OY-OM-2Y-OB.	Egypt
Sids-6	Maya (S) Mou (S)//CMH 74A 592/3/ Sakha 8 *25 D 1002-4sd-3sd-1sd-0sd.	Egypt
ACSAD-925	GEN/3/Gov/AZ//MUS»S»/4/Sannine/Ald»S» ACS-W-9174-10 IZ-5 IZ-0 IZ.	ICARDA
Gemmeiza-10	MAYA74»S»/0N//1160-147/3/BB/GLL/4/CHAT «S»/CROW»S»	Egypt
ACSAD-935	ACSAD 529//Yr/Sprw»S»ACS-W/8023-1IZ-2I Z-0IZ	ICARDA
Line-1	N.S.732/Pim/Veery(S) sd 735- 4sd-1sd 0sd/3/ CM 87688 – 02910P m-5Y-OH-Osy-1M-0Y	Egypt

had been utilized in the single-primer PCR (Supplementary Table S1) as suggested by Klein-Lankhorst et al. (1991). The amplification was performed in a Perkin Elmer 2400 thermal cycler programmed for 5 minutes at 94°C followed by 40 cycles of 1-minute at 94°C, 1-minute at 34°C, 2 minutes at 72°C, using the fastest available transitions between each temperature (ramp time), followed by one cycle of 72°C for 20 minutes; and 4°C thereafter. The annealing temperature varied according to the melting temperature for the lowest primer in the combination. If amplification was weak, the core program increased from 40 to 45 cycles to get a slight increase in the amount of PCR products.A set of 15 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 $MgCl_2$, 0.1 mM dNTPs, 0.8 U of Taq DNA polymerase and 0.3 uM primer in a 25 µL reaction. After initial denaturation at 94°C for 5 min, each cycle consisted of 30 seconds denaturation at 94°C, 45 seconds of annealing at 50°C, 2 minutes extension at 72°C along with 5 minutes extension at 72°C at the end of 40 cycles. The annealing temperature varied according to the melting temperature of each primer. Moreover, if amplification was weak, the core program increased from 40 to 45 cycles to get a slight increase in the amount of PCR products. Allused 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 size of DNA bands on the gel was calculated using 100 bp DNA ladder (GeneRuler 100 bp Plus DNA Ladder, Thermo Fischer Scientific, USA). The genetic similarity among parents was investigated by Nei's genetic distance (Nei 1978). 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).

Crossing among selected parents

Three crosses were performed between the selected genetically diverse six parents, namely, Giza-168, Sids-6, ACSAD-925, Gemmeiza-10, ACSAD-935 and Line-1. Three crosses involving the selected parents *viz.*, Giza-168 x Sids-6 (1st cross), ACSAD-925 x Gemmeiza-10 (2nd cross) and ACSAD-935 x Line-1 (3rd cross) were generated. The F_1 crosses were selfed to produce F_2 populations and backcrossed with their parents to produce B_1 and B_2 populations. Six populations for each cross, P_1, P_2, F_1, F_2, B_1 and B_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 Cd solution at the beginning of the heading stage by a concentration of 30 mg/L Cd ion/liter of water (475 liter/ha). Cadmium sulfate was used as source of cadmium in the present study. The second experiment was used as a control with pure water spraying. Rows were 2.5 m long and 20 cm apart, while a plant-to-plant space was 10 cm. The experiments were irrigated using the common irrigation system in the region, applying surface irrigation. Recommended fertilizers doses for wheat production in the region were applied. Phosphorus, potassium, and nitrogen fertilizers were applied at rates of 75 kg P_2O_5/ha, 100 kg K_2O and 180 kg N/ha. Other agronomic practices, such as weed, disease, and pest control were performed following the recommended agricultural practices for wheat production in the region.

Measurement of 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 content, 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-JarrellAsh, Waltham, MA, USA), (Hart et al. 2005).

Statistical analysis and biometrical assessments

Analysis of variance for all evaluated traits was done using SAS Software. An index of Cd sensitivity (Cd SI) was computed as described by Fisher and Maurer (1978) using the following equation: Cadmium Sensitivity Index (CdSI) $= \frac{[1-(\frac{Ys}{Yp})]}{SI}$ Ys and Yp are the grain yield/plant of each genotype under stress and normal conditions, respectively. SI is stress intensity $= 1 - (\frac{\bar{Y}s}{\bar{Y}p})$, Ys and Yp are averages of grain yield for all genotypes under Cd-stress and non-stress 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 ($MPH_{\%}$) $= \frac{[F1-MP]}{MP} \times 100$. Standard heterosis ($SH_{\%}$) $= \frac{[F1-\text{Check cultivar}]}{\text{Check cultivar}} \times 100$. Inbreeding depression $= \frac{[F1-F2]}{F1} \times 100$, 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 = (VB_2+ VB_1) and the dominance at different loci (F/√ H × D).

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

Genetic diversity among selected parents at molecular level

The reliability of genetic diversity depends mainly on the amount of genetic variability among the used parents. Utilizing molecular markers increases the efficacy of classical plant breeding byassessing the genetic diversity among used parents Abu Hammad et al. 2016; Kumar et al. 2022). The current study investigated genetic diversity using triple-primer RAPD and ISSR markers (Table 1). A total of 440 bands were recorded, in average 35% band per primer/gel, 12% polymorphic, 25% unique bands and 36% polymorphic (with unique), which revealed 70 to 80% polymorphism. 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. 1). The dendrogram displayed genetic diversity among used wheat parents. Based on Triple-RAPD results the evaluated genotypes were classified into diverse four groups. Moreover, ISSR technique frequently utilizes 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 the preliminary screening of six wheat genotypes. However, only fifteen ISSR primers identified intra-specific variation in wheat genotypes produced on average 15 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 a dendrogram utilizing UPGMA. The obtained dendrogram showed different clusters displaying variations in the frequencies of SSR motifs (Fig. 2).

The results of Triple-RAPD and ISSR markers reflected

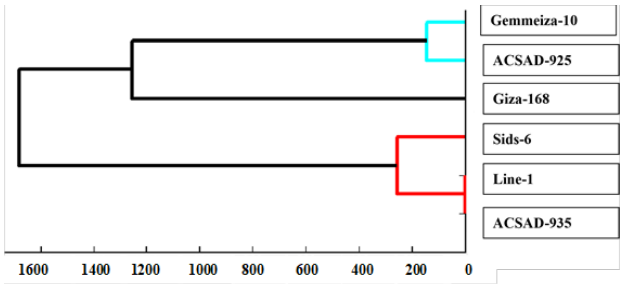

Fig. 1. Dendrogram based on the algorithm of unweighted pair group method with arithmetic averages using RAPD results between in different wheat cultivars

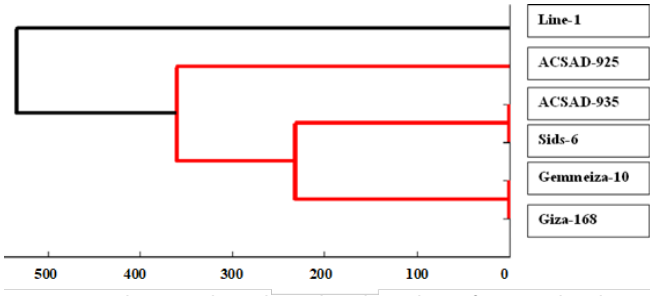

Fig. 2. Dendrogram based on the algorithm of unweighted pair group method with arithmetic averages using ISSR results between in different wheat cultivars in Egypt

the degree of genetic variation among the studied parents. The findings demonstrated relatively high genetic diversity among the parents, which signifies diverse genes. The success of any breeding programme depends on the genetic potential of the genotypes being considered in a breeding programme even for Cd-stress tolerance (Oladzad-Abbasabadi et al. (2018). Accordingly, it is hypothesized that hybridization among the selected parents is expected to release useful genetic diversity upon which selection of promising genotypes depends. Several rersearchers (Pozina et al. 2012; Abu Hammad et al. 2016; Liu et al. 2019; Hou et al. 2021) have advocated that molecular markers potentially distinguish the parents with respect to genetic diversity existing in the parents for improving Cd tolerance.

Heterosis and F_2 deviation

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 displayed positive and significant relative and standard heterosis under both conditions as well as the 1st cross under Cd-stress while 3rd cross exhibited standard heterosis only under Cd-stress (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-stress conditions (Table 2). Desirable heterosis over mid-parents or standard cultivar has been registered for grain yield/plant in the 1st and 2nd cross under both conditions and the 3rd cross only under non-Cd stress conditions (Table

Table 2. Estimates of heterosis, inbreeding depression, dominance and F_2 deviations for evaluated characters of the three wheat crosses under non-Cd-stress and Cd-stress 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-stress 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-stress 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 content (mg Cd/kg DW)			Grain yield per plant (g)			Cd sensitivity index		
	Non-Cd-stress 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-stress 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			

2). On the other hand, negative heterosis in the desired direction was detected for Cd content in the 2nd cross under non-Cd stress and standard heterosis in 1st and 2nd crosses under Cd-stress as well as for Cd sensitivity index in 1st and 2nd crosses for mid-parent heterosis (Table 2). These crosses accumulated lower amounts of Cd in grains rather than the other crosses *i.e.*, 3rd cross under both conditions which accumulated higher amounts of Cd 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 content were recorded by Clarke et al. (1997) and Lin et al. (2016). Similarly, Awaad et al. (2013) recorded significant heterosis for flag leaf area, leaf chlorophyll content, proline content, Cd content and grain yield/plant under both non-Cd stress and Cd-stress conditions.

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 content in the 3rd cross under both conditions. Similarly, inbreeding depression and dominance deviations were significantly positive for proline content in 1st cross and Cd content in 2nd cross under Cd-stress as well as grain yield/plant in 1st and 3rd crosses under non-Cd stress conditions. These results could be discussed on the basis of heterotic effects and dominance and/or dominance × dominance gene effects in the assessed crosses. Conversely, decreasing alleles were involved in the inheritance of Cd content in 1st cross under both conditions and grain yield/plant in 1st and 2nd crosses under Cd-stress conditions. Otherwise, dominance deviation exhibited significantly positive values for grain yield/plant in 2nd cross under non-Cd stress condition and 1st and 2nd crosses under Cd-stress. Moreover, dominance deviation exhibited negative and significant values for leaf chlorophyll content in 3rd cross under non-Cd stressand proline content under

Table 3. Predicted properties of recombinant lines exceeding parental range for evaluated traits of three wheat crosses under non-Cd stress and Cd-stress 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-stress conditions									
m=F_2	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-stress conditions									
m=F_2	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 content (mg Cd/kg DW.)				Grain yield per plant (g)			*Cdsensitivity index*		
	1st cross	2nd cross	3rd cross	1st cross	2nd cross	3rd cross	*1st cross*	*2nd cross*	*3rd cross*
	Non-Cd-stress conditions								
m=F_2	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-stress conditions									
m=F_2	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			
Proportionof inbreds	40.52	16.35	19.77	37.45	7.64	4.85			

m = F_2 mean for each cross, d = Additive genetic components based on mean, D = Additive genetic variance, and proportion of inbredsis the proportion of inbreds falling outside parental range.

Cd-stress 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 contentin 1st cross; grain yield/plant in 2nd cross under both conditions; leaf chlorophyll content in 3rd cross under Cd-stress. Otherwise, it was negative and significant for flag leaf area, Cd content in 3rd cross; proline content in 2nd and 3rd crosses under both conditions, leaf chlorophyll content in 3rd cross under non-Cd stress as well as Cd sensitivity index in 1st and 2nd crosses. The tested crosses exhibited desirable positive F2 deviation for agronomic performance and negative value for Cd content under Cd-stress conditions. Accordingly, these crosses could be expoited for high-yielding and Cd tolerant genotypes with low-grain Cd contents.

Predicting properties of new recombinant lines

Predicted properties of new recombinant lines that fall

Table 4. Predicted properties of recombinant lines exceeding F_1 for evaluated traits of the three wheat crosses under non-Cd-stress and Cd-stress conditions.

Parameters	Flag leaf area (cm^2)			Leaf chlorophyll content (SPAD value)			Proline content (µ moles/g. FW)			Cd content (mg Cd/ kg DW)			Grain yield per plant (g.)			Cdsensitivity index		
	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 cross	2nd cross	3rd cross
Non-Cd-stress 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	0.41	0.39
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	15.63	34.09	34.83
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	1.35	0.83
Cd-stress 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			

h = Dominance genetic component based on mean, D = Additive genetic variance, proportion of inbreds is the proportion of inbreds exceeding F_1, and P Max is best-inbred line.

outside the parental range and exceed F_1 hybrid are presented in Tables 3 and 4. 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 content 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 stress conditions were recorded by 3rd cross for flag leaf area (21.48%) and Cd content (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 the highest percentages of segregants for the Cd sensitivity index (45.62%). On the contrary, under Cd-stress 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 content (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 stress to Cd-stress conditions were assigned for 2nd cross in leaf chlorophyll content and 3rd cross for grain yield/plant, but fluctuated from non-Cd stress to Cd-stress in the other crosses.

The highest proportion of inbreds exceeding F_1 under non-Cd stress 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 content (47.61%), grain yield/plant (36.32%) and Cd sensitivity index (34.83%) (Table 4). Moreover, under Cd-stress the highest proportions exceeding F_1 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 content (41.29%). The best-inbred line (P max) that will have all favorable alleles tended to decrease from non-Cd stress to Cd-stress as a result of Cd effect (Table 4). P max was recorded by 3rd cross for flag leaf area, Cd content and grain yield/plant, while 2nd cross for leaf chlorophyll content and proline content under non-Cd stress. Whereas under Cd-stress, the best-inbred line (P max) was registered by 2nd cross for flag leaf area, leaf chlorophyll content, proline content and Cd content while 3rd cross for grain yield/plant. A similar interpretation was stated by Mather and Jinks (1982) and Awaad (2002) elucidated that a high proportion of recombinants falling outside parental range and exceeding F_1 for grain yield/plant and morpho-physiological traits.

Gene effect and heritability

The nature of gene action and heritability plays an important role in identifying the appropriate breeding method to

Table 5. Measured genetics parameters controlling Cd-stress tolerance in the evaluated traits of three wheat crosses under non-Cd-stress and Cd-stress conditions

Parameter	Flag leaf area (cm^2)			Leaf chlorophyll content (SPAD value)			Proline content (µ moles/g FW)			Cd content (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-stress 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
$F/\sqrt{H \times D}$	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-stress 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
$F/\sqrt{H \times D}$	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

m = Mean for each cross, d = Aadditive genetic component, h = Dominant genetic component, F = Frequency between dominance and recessive alleles in the parental populations, D = Additive genetic variance, H = Dominance genetic variance, h_2 = Heritability estimates from parent-offspring regression, RH = Realized heritability and Gs% = Genetic advance from selection

improve economic traits through breeding programs. Genetic parameters controlling Cdstress tolerance and related traits are presented in Table 5. 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 content in 1st cross under both conditions and 2nd and 3rd crosses under non-Cd stress 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 content suggests the opportunity of breeding consistently low-Cd wheat cultivars crossways environments.

The dominance gene effect [h] indicating 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 stressand 2nd and 3rd under Cd-stress; Cd content in 1st cross under non-Cd stress and 2nd and 3rd crosses under Cd-stress and Cd sensitivity index in 1st and 3rd crosses, reflecting potency ratio h/d was more than unity (Tables 5 and 6). 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 content in 2nd and 3rd crosses under non-Cd stress condition as well as for proline content in 1st cross and Cd content in the three crosses under Cd-stress 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

Table 6. Estimated genetic parameters for Cd sensitivity index of three wheat crosses under non-Cd-stress and Cd-stress conditions

Parameter	1st cross	2nd cross	3rd 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

m = Mean for each cross, d = Additive genetic component, h = Dominant genetic component, F = Frequency between dominance and recessive alleles in the parental populations, D = Aadditive genetic variance, H = Dominance genetic variance, h_2 = Heritability estimates from parent-offspring regression, RH = Realized heritability and Gs% = Genetic advance from selection

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.

F values indicate the frequency between dominance and recessive alleles in the parental populations. Also, F/√(H×D) also provided evidence that the dominance at different loci is 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 content 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 stress 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 cross under non-Cd stress 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 5 and 6. Estimates from parent-offspring regression (h^2) were high (<50%) for flag leaf area, leaf chlorophyll content, proline content and Cd content in most studied crosses under both conditions. While it was moderate for flag leaf area and leaf chlorophyll content under Cd-stress, 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 content under non-Cd stress 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 a percentage of the population mean was high for proline content, Cd content 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. High heritability and genetic advance for flag leaf area, leaf chlorophyll content, proline content, and Cd content reveal the considerable improvement could be brought in through the selection of promising genotypes under d-stress conditions. Clarke et al. (1997) and Awaad et al. (2010) have exploited the genetic variability created by hybridization through selection based on morpho-physiological traits and Cd contents for improving Cd tolerance.

Supplementary material

Supplementary Table S1 is provided, which can be accessed online www.isgpb.org.

Authors' contributions

Conceptualization of research (HAA, AMA, AMM, ESAM, EM); Designing of the experiments (HAA, AMA, AMM, ESAM, EM); Contribution of experimental materials (HAA, AMA, AMM, ESAM, EM); Execution of field/lab experiments and data collection (HAA, AMA, AMM, ESAM, EM); Analysis of data and interpretation (HAA, AMA, EM); Preparation of the manuscript (HAA, AMA, EM).

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Supplemenatary Table S1. 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		