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Localization and gene action studies for kernel iron and zinc concentration in groundnut (*Arachis hypogaea* L.)

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Abstract Iron (Fe) and zinc (Zn) are important micronutrients for human health and well-being. Groundnut kernels are good sources of Fe and Zn. Localization studies of Fe and Zn in the kernel tissues of ten diverse groundnut genotypes revealed that, cotyledons contribute nearly 85–90% of total Fe and Zn in comparison to seed coat and embryo on dry matter basis. Generation mean analysis revealed the predominant role of additive gene action for kernel Fe and Zn concentration in the cross ICGV 06,040 × ICGV 87,141, and both additive and additive × additive interaction in the cross ICGV 06,099 × ICGV 93,468. Duplicate epistasis was observed for kernel Fe and Zn concentrations in both the crosses. For yield parameters, pod yield per plant and 100-kernel weight, dominance gene action was significant. Additive × additive interaction was also found to be significant for these traits which can be fixed through selection. For days to maturity, additive, dominance, additive × additive and dominance × dominance interactions were significant. The study involved six generations of two crosses involving parents with contrasting kernel Fe and Zn concentrations and was conducted for five economically important traits using a six-parameter model. There was significant positive association between kernel Fe and Zn concentration in both the crosses indicating possibility of simultaneous improvement. Absence of association of kernel Fe and Zn concentration with pod yield per plant will enable the development of high pod-yielding varieties with elevated levels of kernel Fe and Zn concentration.

Keywords Additive · Dominance · Generation mean analysis · Groundnut · Iron · Zinc

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Introduction

Micronutrients deficiencies are predicted to affect half of the world's population, especially women and preschool children in the developing world (UNSCN 2004). Among the micronutrients, iron (Fe) and zinc (Zn) deficiencies alone affect over three billion people around the globe (WHO 2002). Biofortification or breeding crop plants for higher micronutrient concentration is one of the most successful interventions for addressing the issues pertaining to micronutrient deficiencies. It aims to develop micronutrient-dense crops using the best traditional breeding practices and modern biotechnology.

Groundnut (*Arachis hypogaea* L.) or peanut is one of the important food, oil seed and fodder crop of the world. It is utilised for human consumption as a vegetable oil and food crop, as a green manure for improving soil fertility and as fodder for livestock. Groundnut is cultivated all around the world with a total production of 46 million tons. China ranks first in groundnut production with a total production of 17 million tons followed by India (9.47 million tons) (FAOSTAT 2014). Groundnut is highly valued as a rich source of energy contributed by oil (48–50%), protein (25–28%) and carbohydrates (10–20%) in the kernels. In addition, groundnut kernels also contain antioxidants, vitamins and are rich in mono-unsaturated fatty acids (Janila et al. 2013). They contain vitamin E and many important B-complex group of vitamin like thiamin, pantothenic acid, vitamin B-6 and niacin. Of the 20 minerals necessary for normal body growth and maintenance, seven, including Fe and Zn are present in groundnut. Developing countries, where micronutrient deficiencies are widespread, contribute world's maximum groundnut area and production (FAOSTAT 2011). Groundnut is used as food crop, to prepare food supplements to infants and elderly people and ready-to-use-therapeutic food (RUTF) products to treat acute malnutrition under different programs of UNICEF and others. The food products based on groundnut meet the key criteria of availability, affordability, acceptability, nutritional quality and business interest, the necessary criteria for foods to contribute to reduce under-nutrition. Donors including USAID consider groundnut as one of the important crops for reducing malnutrition. Projects like 'SPRING nutrition' in Ghana and 'Groundnut Scaling' in Mali, Ghana and Nigeria promote home consumption of groundnut for enhanced nutrition. The RUTF products based on groundnut are low cost and proven solutions in treating malnutrition among children and women. 'PlumpyNut', a RUTF is used by UNESCO to treat acute malnutrition in children in Niger. Groundnut and its products can contribute significantly towards reduction of protein-energy and micronutrient malnutrition (Janila et al. 2014).

The localization of Fe and Zn in the seed tissues varies depending on the crop species. For example, in monocots such as rice the highest Fe concentration was observed in aleuron layers, integument, and in the scutellum; whereas in dicots Fe is mainly stored within the embryo. In rice the high Fe containing tissues are discarded during processing while in dicots raising the Fe content might be associated with a possible damage of the embryo by toxic Fe concentration (Grillet et al. 2014). Thus, understanding of the inherent processes involved in mineral localization is useful to devise breeding strategies that ensure the success of biofortification. For example in rice, to overcome the processing loss breeding strategies focused on increasing endosperm Fe content in order to enhance Fe bioavailability (Bashir et al. 2011). In the present study an attempt was made to identify the distribution pattern of Fe and Zn concentration on groundnut kernel tissues viz. seed coat, cotyledon and embryo and to identify tissues responsible for maximum accumulation of these minerals.

Significant genetic variations in the seed concentrations of Fe and Zn was reported in several crops such as rice (Anuradha et al. 2012), wheat (Morgounov et al. 2007), maize (Maziya-Dixon et al. 2000), sorghum (Kumar et al. 2009) and groundnut (Upadhyaya et al. 2012; Janila et al. 2014). The nature of inheritance and presence of quantitative trait loci (QTL) for Fe and Zn concentration were also reported in some crops. In rice, additive and dominant gene effect besides environmental effect was documented for grain Fe and Zn concentrations (Gregorio 2002). In common bean seeds, additive allelic interaction for Zn concentration, and both additive and dominance component for Fe concentration was reported (Silva et al. 2013). Additive gene action and additive × dominance epistasis for kernel Fe, and dominance and additive × additive epistasis for kernel Zn concentration was observed in maize seeds (Chakraborti et al. 2009). In sorghum, non-additive gene action was predominant for Fe concentration, while additive effects were important for grain Zn concentration (Kumar et al. 2013). Information on the nature of gene action associated with important target traits is crucial to decide the breeding procedure to be followed. Generation mean analysis (GMA) is often used to estimate the components of gene action (additive, dominance effects and interactions) of individual traits. In groundnut, GMA was carried out to understand the gene action for yield and its contributing characters (Shobha et al. 2010;

Venuprasad et al. 2011), resistance to late leaf spot (Janila et al. 2013) and surrogate traits for water-use-efficiency (Janila et al. 2015). Though significant variability for kernel Fe and Zn concentration was reported in groundnut (Asibuo et al. 2000; Janila et al. 2014) mechanism of gene action associated with these traits was not reported yet. Hence, the present study also aimed at understanding the nature of gene action governing the kernel Fe and Zn concentrations.

Materials and methods

Localization studies for kernel Fe and Zn concentration

Ten genotypes viz., ICGV 06,099, ICGV 93,468, ICGV 91,114, TAG 24, ICGV 00,440, ICGV 02,266, ICGV 05,155, ICGV 06,420, ICGV 06,040 and ICGV 87,141, each differing in their kernel Fe and Zn concentrations was used in the present study. From each entry 100 g of kernels was weighed after which each kernel was manually separated into seed coat, cotyledons and embryo. Each tissue from each sample was separately ground into fine powder and used for estimating Fe and Zn concentration using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method.

Plant material and experiment design

Four generations viz., F_1, F_2 and first back cross generations, B_1 with first parent and B_2 with second parent along with the two parents P_1 and P_2 constituted the six-generations. These generations were derived from two crosses, ICGV 06,040 × ICGV 87,141 and ICGV 06,099 × ICGV 93,468 and evaluated in a compact family block design in precision fields on Alfisol (clayey-skeletal, mixed, iso-hypothermic family of Udic Rhodustalfs) at Patancheru (17°53′ N, 78°27′ E, and 545 m altitude), India during 2013–14 post-rainy season. The two parents in the cross had contrasting kernel Fe and Zn concentration (Table 1). The experimental block comprised of one row each of P_1, P_2 and F_1, two rows each of B_1 and B_2, and eight rows of F_2. Each row is of 2 m length with a spacing of 30 cm between rows and 10 cm between the plants in a row. Standard package of practices that included application of 60 kg P_2O_5 as basal application, seed treatment with mancozeb @ 2 g kg^{-1} of seed and imidachloprid @ 2 ml kg^{-1} of seed, pre-emergence application of pendimethalin @ 1 kg active ingredient ha^{-1}, irrigation soon after planting and subsequently as and when needed, application of gypsum @ 400 kg ha^{-1} at the peak flowering and protection against insect pests and diseases as per the requirement was followed to raise a healthy crop. The observations were recorded on days to emergence, days to 75% flowering, days to maturity, 100-kernel weight (g), shelling percentage, sound mature kernel percentage, pod yield per plant (g), kernel Fe and Zn concentrations. The Fe and Zn concentration of parents and different generations were estimated using the ICP-OES method.

Estimation of kernel Fe and Zn concentration

Kernel Fe and Zn concentrations from individual samples were estimated using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method (Prodigy High Dispersion ICP, TELEDYNE Leeman labs, USA). About 0.2–0.3 g of oven-dried ground sample was weighed and transferred into labelled tube. To this, 1.5–2.0 ml of nitric acid was added followed by 0.5 ml of Hydrogen Peroxide (H_2O_2). The tube was then closed and allowed to stand overnight at room temperature, after which the contents were transferred to heating blocks set at 80^{0}C. The cap was loosened to allow release of pressure during the reaction process. After 30-min of reaction initiation, the temperature of heating block was increased to 125 °C and heat digestion was continued for another 2-h. The sample tubes were cooled and the volume was made up to 25 ml using distilled water. The tubes were vortexed for 1–2 min and the supernatant was collected and used for estimating Fe and Zn concentration through ICP-OES. The samples used in this method are non-defatted, and it is expected that the estimations based on defatted samples will be higher than the non-defatted samples (Janila et al. 2014).

Statistical analysis

The mean values from individual plant data were estimated for all the studied traits, for each generation separately and used to compute variance (ANOVA) for compact family design as described by Panse and

Table 1 Pedigree and characteristics of the groundnut genotypes used as parents in the crossing program

Parental line	Pedigree	Characteristics
ICGV 06,040	[{(ICGS 35 × NC Ac 1705) × CS 16-B2-B2} × {(NC Ac 343 × (Dh. 3–20 × Robut 33–1)} × {(NC Ac 343 × (Dh. 3–20 × Robut 33–1)}]	Spanish bunch, medium duration, slight reticulated pods, tan colour and medium size seed. High kernel iron and zinc concentration (Janila et al. 2014)
ICGV 87,141	(TMV 10 × Chico)	Virginia bunch, medium duration, slight reticulated pods, tan colour and medium size seed. Low kernel iron and zinc concentration (Janila et al. 2014)
ICGV 06,099	[{(ICGS 35 × NC Ac 1705) × CS 16-B2-B2} × {(NC Ac 343 × (Dh. 3–20 × Robut 33–1)} × {(NC Ac 343 × (Dh. 3–20 × Robut 33–1)}]	Spanish bunch, medium duration, slight reticulated pods, tan colour and medium size seed. High kernel iron and zinc concentration (Janila et al. 2014)
ICGV 93,468	[(ICGS 44 × TG 2E) × {ICGS 30 × (TMV 10 × Chico)}]	Spanish bunch, short duration, medium reticulated pods, red colour and small to medium size seed. Low kernel iron and zinc concentration (Janila et al. 2014)

Sukhatme (1985). The traits showing significant difference between crosses and among the generations were further subjected to GMA. Six generations, the parents, F_1, F_2, B_1 and B_2 were used to fit in simple additive-dominance model in the generation means approach. Scaling tests for five traits of both the crosses were performed to test the adequacy of the additive-dominance model (Mather 1949). Six-parameter model proposed by Hayman (1958) was used to estimate mean (m), additive (d) and dominance (h) effects, and those resulting from their interactions, additive × additive (i), additive × dominance (j) and dominance × dominance (l) effects. The validity of the additive-dominance model for the scaling tests and non-allelic interactions were examined using WINDOSTAT 8.5 software. Correlation between the traits was carried out using GENSTAT 14th edition software.

Results

Localization of Fe and Zn in groundnut kernels

The concentration of Fe and Zn in different kernel tissues viz., seed coat, cotyledons and embryo of the ten tested genotypes is given in Table 2. Estimates of kernel Fe and Zn concentration in different kernel tissues revealed that seed coat contributed maximum towards total kernel Fe accumulation followed by embryo and cotyledon; whereas for kernel Zn concentration, embryonic portion contributed maximum followed by cotyledon and seed coat. The Zn contributed by the embryonic portion alone was much higher (65–70%) than the total contribution of both cotyledons and seed coat.

Among the ten tested genotypes, seed coat of ICGV 02,266 contributed maximum for kernel Fe concentration (527.36 mg kg^{-1}) followed by TAG 24 (272.11 mg kg^{-1}), ICGV 00,440 (261.84 mg kg^{-1}) and ICGV 05,155 (246.12 mg kg^{-1}). In contrast, ICGV 06,040 recorded higher Fe concentration in its embryo (90.50 mg kg^{-1}) than in seed coat (73.42 mg kg^{-1}), indicating variation among genotypes with regards to Fe localization. The Fe present in seed coat is not available for consumption as it will be removed during processing in case of confectionery groundnuts. Thus, the presence of high Fe in the embryo rather than seed coat indicates that the genotype ICGV 06,040 can be suitably used as parent in breeding program to develop high Fe containing lines or varieties. For kernel Zn concentration, embryo portion of ICGV 06,099 recorded highest value of 138.92 mg kg^{-1} followed by ICGV 06,040 (128.38 mg kg^{-1}), ICGV 00,440 (94.83 mg kg^{-1}) and TAG 24 (87.50 mg kg^{-1}). However, considering each kernel part weight *i.e.* seed coat, cotyledons and embryo, cotyledons contribute more than 90% of total weight of the kernel. Hence, the proportional contribution of cotyledons to the total kernel Fe and Zn will be more than seed coat and embryo ($\sim$ 3–4%).

Table 2 Localization studies on iron and zinc concentration in kernel tissues of ten groundnut genotypes

Sl No	Genotype	Kernel tissue	Proportional weight	Fe (ppm)	Zn (ppm)	Fe per 100 g	Zn per 100 g	Proportional weight towards total Fe	Proportional weight towards total Zn	Percentage contribution of Fe	Percentage contribution of Zn
1	ICGV 06,099	Seed coat	3.6	93.69	39.17	9.37	3.92	33.73	14.10	14.01	3.33
		Cotyledons	91.8	17.77	37.70	1.78	3.77	163.14	346.08	67.78	81.61
		Embryo	4.6	95.25	138.92	9.52	13.89	43.81	63.90	18.20	15.07
2	ICGV 93,468	Seed coat	3.3	73.94	16.57	7.39	1.66	24.40	5.47	13.64	1.54
		Cotyledons	92.4	14.23	33.81	1.42	3.38	131.51	312.43	73.50	87.92
		Embryo	4.3	53.53	87.11	5.35	8.71	23.02	37.46	12.86	10.54
3	ICGV 91,114	Seed coat	3.6	133.49	11.19	13.35	1.12	48.06	4.03	21.20	1.32
		Cotyledons	92.1	16.54	28.84	1.65	2.88	152.31	265.66	67.18	86.75
		Embryo	4.3	61.30	84.97	6.13	8.50	26.36	36.54	11.63	11.93
4	TAG 24	Seed coat	3.3	272.11	18.10	27.21	1.81	89.80	5.97	33.30	2.04
		Cotyledons	92.4	16.68	26.96	1.67	2.70	154.10	249.10	57.14	85.10
		Embryo	4.3	59.94	87.50	5.99	8.75	25.77	37.63	9.56	12.85
5	ICGV 00,440	Seed coat	3.0	261.84	21.86	26.18	2.19	78.55	6.56	32.80	1.78
		Cotyledons	93.8	15.45	35.39	1.55	3.54	144.96	331.92	60.52	89.99
		Embryo	3.2	50.02	94.83	5.00	9.48	16.00	30.35	6.68	8.23
6	ICGV 02,266	Seed coat	3.0	527.36	14.88	52.74	1.49	158.21	4.46	46.83	1.61
		Cotyledons	93.3	16.71	25.88	1.67	2.59	155.95	241.49	46.16	87.11
		Embryo	3.7	64.10	84.54	6.41	8.45	23.72	31.28	7.02	11.28
7	ICGV 05,155	Seed coat	3.2	246.12	19.18	24.61	1.92	78.76	6.14	36.86	2.02
		Cotyledons	92.3	12.07	28.24	1.21	2.82	111.41	260.63	52.15	85.81
		Embryo	4.5	52.17	82.14	5.22	8.21	23.48	36.96	10.99	12.17
8	ICGV 06,420	Seed coat	4.5	68.71	21.67	6.87	2.17	30.92	9.75	15.14	3.40
		Cotyledons	91.5	16.74	27.24	1.67	2.72	153.18	249.27	75.03	86.84
		Embryo	4.0	50.17	70.02	5.02	7.00	20.07	28.01	9.83	9.76
9	ICGV 06,040	Seed coat	4.0	73.42	37.80	7.34	3.78	29.37	15.12	12.66	3.58
		Cotyledons	90.4	16.81	37.10	1.68	3.71	151.98	335.35	65.50	79.40
		Embryo	5.6	90.50	128.38	9.05	12.84	50.68	71.89	21.84	17.02
10	ICGV 87,141	Seed coat	4.0	52.00	25.57	5.20	2.56	20.80	10.23	13.24	3.48
		Cotyledons	91.7	12.57	27.24	1.26	2.72	115.27	249.78	73.38	84.99
		Embryo	4.3	48.89	78.82	4.89	7.88	21.02	33.89	13.38	11.53

Mean performance of traits across generations

The mean performance of six generations, P_1, P_2, F_1, F_2, B_1 and B_2 of two crosses for kernel Fe and Zn concentrations and other traits is given in Table 3. For days to 75% flowering, variation between the generations was not observed. The mean performance across generations varied from 48 to 50 days for the cross ICGV 06,040 × ICGV 87,141, and from 47 to 48 days for the cross ICGV 06,099 × ICGV 93,468. However, significant variation was observed for days to maturity (Fig. 1). The mean performance for days to maturity across the generations varied from 142 to 159 days in the cross ICGV 06,040 × ICGV 87,141, and from 133 to 159 days in the cross ICGV 06,099 × ICGV 93,468. In both the crosses, P_2 parents, ICGV 87,141 and ICGV 93,468 were early maturing, followed by B_2 generations, which were crossed to P_2 parent suggesting possible contribution of alleles for early maturity from P_2 parents in the studied crosses. Significant variation for days to maturity, despite absence of variation for duration for 75% flowering, suggests importance of pod-filling duration to total maturity duration. Pod-filling duration indicates the stage from fertilisation to formation of fully filled mature pods. For breeding program across Asia and Africa, early-maturity is an important target trait for adaptation to short growing season and/or as escape mechanism from end-of-season drought. Observations from the present study suggest the possibility of using pod filling duration as one of the useful criteria for selecting early-maturing types.

Among the generations of the cross ICGV 06,040 × ICGV 87,141, 100-kernel weight (HKW) varied from 35 to 45 g, and from 36 to 46 g in the cross, ICGV 06,099 × ICGV 93,468. Highest mean HKW of 45 g was recorded by both P_1 and B_1 generations in the cross ICGV 06,040 × ICGV 87,141, and 46 g by P_1 and F_1 in the cross ICGV 06,099 × ICGV 93,468 (Fig. 2). For shelling percentage the mean performance varied from 58 to 75% in the cross ICGV 06,040 × ICGV 87,141, and the highest shelling percentage was observed in F_1 (75%) followed by P_1 (72%). No significant variation was observed for shelling percentage in the cross ICGV 06,099 × ICGV 93,468. Variation was not observed for sound mature kernel percentage (SMK) in both the crosses. Significant variation was observed for pod yield per plant in both the crosses, and mean performance varied from 25 to 40 g in ICGV 06,040 × ICGV 87,141, and from 27 to 38 g in ICGV 06,099 × ICGV 93,468. Highest pod yield per plant was recorded for B_1 generation of the cross ICGV 06,040 × ICGV 87,141, while F_1 was the best performer in the cross ICGV 06,099 × ICGV 93,468 (Fig. 2).

The parents of both the crosses showed variation for kernel Fe and Zn concentration (Table 1). The high parents (ICGV 06,040 and ICGV 06,099) had 33 and 25 mg kg^{-1} kernel Fe, while the low parents (ICGV 87,141 and ICGV 93,468) had 25 and 21 mg kg^{-1}, respectively. For kernel Zn, high parents (ICGV 06,040 and ICGV 06,099) had 50 and 36 mg kg^{-1}, while the low parents (ICGV 87,141 and ICGV 93,468) had 36 and 30 mg kg^{-1}, respectively. All values of Fe and Zn are based on non-defatted samples. The mean performance for kernel Fe and Zn concentration varied from 20 to 42 mg kg^{-1} and 23 to 60 mg kg^{-1}, respectively in the cross ICGV 06,040 × ICGV 87,141, and from 16 to 49 mg kg^{-1} and 22 to 61 mg kg^{-1}, respectively in the cross ICGV 06,099 × ICGV 93,468. Parent, P_1 recorded highest value for both kernel Fe and Zn concentration across the generations in the cross ICGV 06,040 × ICGV 87,141, while both P_1 and B_1 showed similar performance in the cross ICGV 06,099 × ICGV 93,468 (Fig. 2).

In order to test the performance of nine characters for comparison of crosses and generations of each cross, analysis of variance (ANOVA) was performed and the results are presented in Table 4. The mean squares from ANOVA revealed significant differences among the crosses for five traits viz., days to maturity, 100-kernel weight, pod yield per plant, kernel Fe and Zn concentrations which indicated that considerable amount of variability was present between the crosses and among the generations of the two crosses for these traits. So, further genetic analyses of generation means was carried out for five traits in both the crosses.

Generation mean analysis by scaling tests

To understand the mechanism of gene action, scaling test was performed using the mean measurements of six generations for five traits, days to maturity, 100-kernel weight, pod yield per plant, kernel Fe and kernel Zn concentrations in both crosses (Table 5). Significant differences were observed for all five traits

Table 3 Means and standard errors (±) for nine characters in six generations of each of the two crosses of groundnut

Trait	P_1		P_2		F_1		F_2		B_1		B_2		S.Em. ±	C.D. (P = 0.05)
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range		
Cross: ICGV 06,040 × ICGV 87,141														
DE	11.27	11.0–12.0	15.38	13.0–18.0	12.20	10.00	14.34	11.0–17.0	15.25	12.0–17.0	15.80	11.0–17.0	1.01	3.17
DF	47.94	47.0–51.0	49.61	48.0–53.0	48.80	46.0–51.0	49.83	43.0–56.0	49.46	46.0–55.0	48.37	45.0–56.0	0.49	NS
DM	159.00	159.0	142.00	142.00	159.00	159.00	158.34	142.0–159.0	158.33	157.0–159.0	155.81	142.0–159.0	1.34	4.21
HKW (g)	44.53	31.4–52.3	34.82	30.2–51.5	43.94	31.0–54.5	43.00	22.7–73.9	44.54	22.2–68.8	36.49	23.1–57.1	1.82	5.75
SHP (%)	72.54	64.0–85.4	57.74	46.9–65.4	75.40	57.0–87.9	66.07	34.9–86.2	61.05	44.5–76.6	65.51	45.6–85.9	1.78	5.62
SMK (%)	64.70	39.2–82.1	55.24	27.6–70.9	60.85	34.2–77.9	67.50	16.4–95.7	54.38	19.6–86.7	68.88	36.8–84.8	4.54	NS
PY(g)	24.82	15.2–45.7	31.09	16.5–53.8	31.67	14.5–53.0	31.40	7.6–86.3	39.99	15.4–100.3	34.12	11.0–105.7	2.63	8.28
KIC (mg/kg)	33.32	29.2–38.6	25.54	21.7–27.7	28.49	23.7–32.2	28.38	20.1–39.1	29.42	20.4–41.4	31.49	21.0–42.2	0.89	2.81
KZC (mg/kg)	50.91	45.9–57.4	36.05	27.7–41.9	40.27	28.2–55.2	39.80	22.7–57.9	42.46	27.6–54.2	41.98	27.1–60.3	1.70	5.35
Cross: ICGV 06,099 × ICGV 93,468														
DE	11.47	11.0–12.0	10.58	10.0–11.0	11.25	11.0–12.0	11.26	10.0–14.0	13.70	12.0–17.0	11.80	11.0–13.0	0.33	1.04
DF	47.42	46.0–48.0	46.91	46.0–49.0	46.95	46.0–49.0	47.74	43.0–53.0	47.17	46.0–51.0	47.44	42.0–52.0	0.35	NS
DM	159.00	159.00	133.00	133.00	147.67	142.0–159.0	156.08	142.0–159.0	155.76	142.0–159.0	136.53	133.0–159.0	2.54	8.00
HKW (g)	46.32	26.0–60.5	44.78	39.5–54.5	46.37	30.0–67.4	36.43	26.0–77.7	45.03	28.6–58.7	44.33	23.2–63.6	1.83	5.77
SH (%)	60.17	45.3–64.3	61.26	47.3–77.5	58.98	41.7–73.3	65.32	38.9–90.1	62.29	35.3–78.7	62.77	28.8–89.7	3.09	NS
SMK (%)	65.18	43.7–75.9	57.86	53.4–65.4	67.88	48.4–76.2	71.79	32.4–90.9	67.34	41.3–86.4	58.28	19.6–91.4	2.67	NS
PY(g)	30.71	20.1–66.9	26.77	16.1–46.7	37.51	18.5–68.6	30.82	4.7–91.3	33.50	9.4–66.7	35.90	9.1–71.7	1.81	5.70
KIC (mg/kg)	25.49	20.9–31.1	20.83	15.8–22.6	21.95	18.7–31.1	25.19	17.3–48.9	26.25	16.2–41.2	24.07	17.6–35.2	1.19	3.74
KZC (mg/kg)	36.58	35.0–54.6	30.39	24.5–35.9	32.01	21.9–41.2	35.08	23.4–60.8	37.27	23.7–50.6	32.91	25.0–42.1	1.86	5.87

NS non significant, *DE* days to emergence, *DF* days to 75% flowering, *DM* days to maturity, *HKW* 100-kernel weight, *SH* shelling percentage, *SMK* sound mature kernel percentage, *PY* Pod yield/plant, *KIC* Kernel iron concentration, *KZC* Kernel zinc concentration

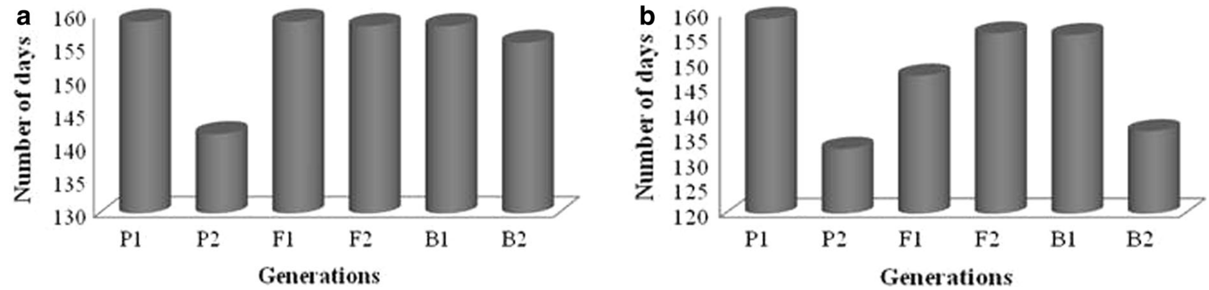

Fig. 1 Mean performance of different generations of groundnut for days to maturity **a** ICGV 06,040 × ICGV 87,141 (5.9 cm (H) × 10.8 cm (W)), **b** ICGV 06,099 × ICGV 93,468 (5.9 cm (H) × 10.9 cm (W)). Parent 1 (P1), Parent 2 (P2), First filial generation (F1), Second filial generation (F2), Back cross 1 (B1) and Back cross 2 (B2)

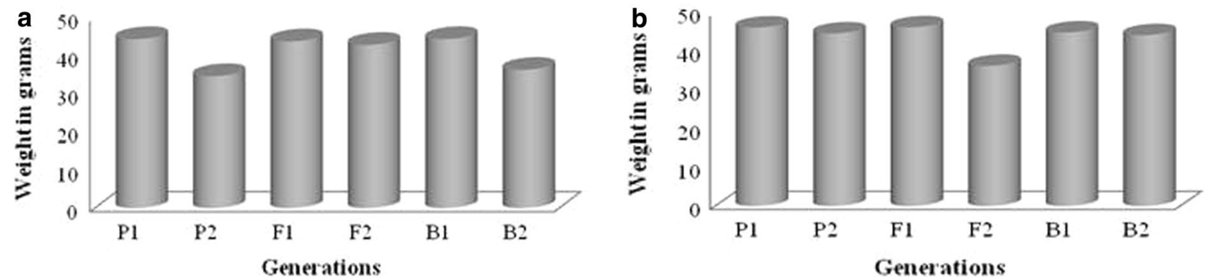

Fig. 2 Mean performance of different generations of groundnut for hundred kernel weight **a** ICGV 06,040 × ICGV 87,141 (5.7 cm (H) × 10.8 cm (W)), **b** ICGV 06,099 × ICGV 93,468 (5.7 cm (H) × 10.9 cm (W)). Parent 1 (P1), Parent 2 (P2), First filial generation (F1), Second filial generation (F2), Back cross 1 (B1) and Back cross 2 (B2)

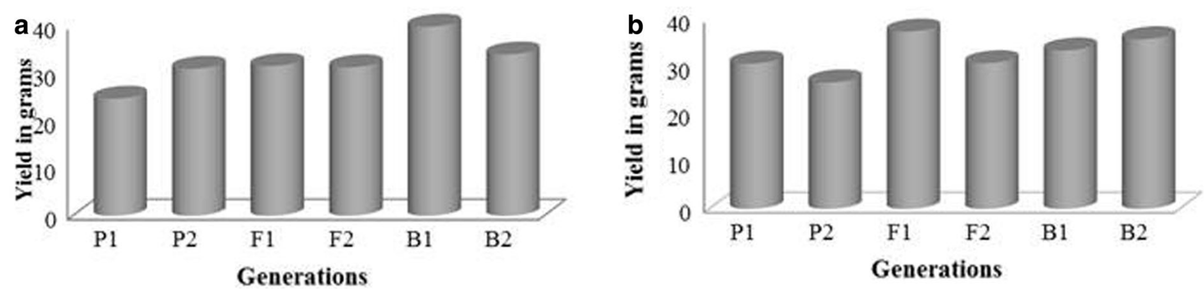

Fig. 3 Mean performance of different generations of two crosses of groundnut for pod yield/plant **a** ICGV 06,040 × ICGV 87,141 (5.3 cm (H) × 10.4 cm (W)), **b** ICGV 06,099 × ICGV 93,468 (5.3 cm (H) × 10.4 cm (W)). Parent 1 (P1), Parent 2 (P2), First filial generation (F1), Second filial generation (F2), Back cross 1 (B1) and Back cross 2 (B2)

in both the crosses for the scaling parameters A, B and/or C, suggesting the presence of non-allelic interactions, and inadequacy of additive-dominance model in explaining the gene action mechanism.

As the scaling tests revealed significant differences for days to maturity, 100-kernel weight, pod yield per plant, kernel Fe and Zn concentration in both the crosses, the six parameter model was used to identify the epistatic interactions involved in governing the above mentioned traits. The parameters viz., mid parental effect (m), additive (d) and dominance (h) effects and interactions viz., additive × additive (*i*), additive × dominance (*j*) and dominance × dominance (*l*) of the two crosses under study are presented

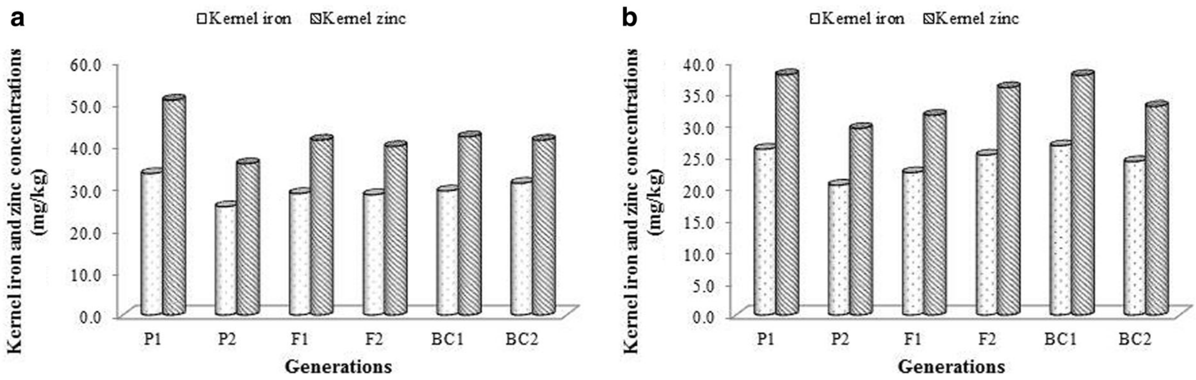

Fig. 4 Mean performance of different generations of two crosses of groundnut for kernel iron and zinc concentrations **a** ICGV 06,040 × ICGV 87,141 (5.7 cm (H) × 10.5 cm (W)), **b** ICGV 06,099 × ICGV 93,468 (5.7 cm (H) × 10.4 cm (W)). Parent 1 (P1), Parent 2 (P2), First filial generation (F1), Second filial generation (F2), Back cross 1 (B1) and Back cross 2 (B2)

Table 4 Analysis of variance (ANOVA) for different characters in two crosses in groundnut

Source of variation	df	Days to maturity	100-kernel weight (g)	Pod yield/ plant (g)	Kernel iron concentration (mg/kg)	Kernel zinc concentration (mg/kg)
Analysis of variance between crosses						
Rep	2	1.99	0.53	0.40	0.07	1.45
Cross	1	24.2720*	3.3856*	2.783336*	6.881878*	14.65614*
Error	2	0.68	0.18	0.06	0.12	0.54
Analysis of variance between generations within crosses						
ICGV 06,040 × ICGV 87,141						
Rep	2	6.33	7.99	7.71	6.23	52.95*
Gen	5	133.75**	57.56**	44.05**	21.17**	72.76**
Error	10	5.36	9.99	7.58	2.38	8.66
ICGV 06,099 × ICGV 93,468						
Rep	2	88.98*	11.04	7.64	0.39	18.61
Gen	5	325.11**	42.10*	62.20**	16.80*	36.09*
Error	10	19.36	10.05	2.45	4.22	10.41

Rep replication, *Gen* generation, *df* degree of freedom

* and ** indicates significance at 5% and 1% probability levels, respectively

in Table 6. In both the crosses, the mid-parental values were highly significant and positive for all the traits except for pod yield per plant.

Gene action governing pod yield and associated traits

Positive significant dominance × dominance interaction and additive gene action, and negative significant dominance gene action and additive × additive interaction were observed for days to maturity in both the crosses. The higher magnitude of dominance effects in both the crosses indicated the major role played by dominance components in determining the maturity duration followed by additive gene action. Both, dominance and dominance × dominance component had opposite signs, indicating the involvement of duplicate epistatic effects. So for earliness, selection will be effective in early generations as negative significance was observed for dominance and additive × additive component of epistasis. In both the crosses, dominance × dominance component was

Table 5 Results of scaling tests for five agronomic traits including kernel iron and zinc concentrations in both the crosses evaluated during post-rainy season, 2013–14

Scaling test	Days to maturity	100-Kernel weight (g)	Pod yield/plant (g)	Kernel iron concentration (mg/kg)	Kernel zinc concentration (mg/kg)
ICGV 06,040 × ICGV 87,141					
A	− 1.302 ± 0.28**	4.336 ± 3.90	23.489 ± 6.96**	− 2.976 ± 1.57	− 6.271 ± 2.82*
B	− 7.650 ± 2.27**	− 15.835 ± 3.17**	5.478 ± 7.79	8.949 ± 1.80**	7.636 ± 3.25*
C	− 2.628 ± 0.91**	− 0.792 ± 4.42	6.346 ± 8.62	− 2.335 ± 1.71	− 8.305 ± 4.09*
ICGV 06,099 × ICGV 93,468					
A	6.401 ± 2.91*	− 0.795 ± 3.75	− 7.855 ± 5.16	5.051 ± 1.67**	5.943 ± 2.46*
B	− 9.417 ± 6.48	− 3.40 ± 3.39	5.664 ± 5.53	5.371 ± 1.77**	3.421 ± 2.04
C	37.528 ± 4.08**	11.891 ± 2.25*	− 19.70 ± 7.32*	10.550 ± 2.30**	12.197 ± 3.16**

* and ** indicates significance at 5% and 1% probability levels, respectively

Table 6 Results of genetic components for five agronomic traits including kernel iron and zinc concentrations in both the crosses evaluated during post-rainy season, 2013–14

Genetic component	Days to maturity	100-Kernel weight (g)	Pod yield/plant (g)	Kernel iron concentration (mg/kg)	Kernel zinc concentration (mg/kg)
ICGV 06,040 × ICGV 87,141					
m	186.299 ± 6.84**	59.984 ± 4.67**	13.210 ± 7.60	23.294 ± 0.36**	36.324 ± 2.93**
d	12.006 ± 0.72**	− 1.193 ± 1.20	1.972 ± 1.90	2.334 ± 0.703**	3.096 ± 0.88**
h	− 82.191 ± 20.17**	− 32.293 ± 12.61*	46.154 ± 21.03*	8.957 ± 6.56	2.220 ± 8.03
i	− 40.543 ± 6.80**	− 16.085 ± 4.51**	15.537 ± 7.36*	− 0.128 ± 2.32	− 2.834 ± 2.79
j	7.909 ± 3.35	1.303 ± 2.11	− 4.371 ± 3.58	− 0.160 ± 1.15	1.261 ± 1.43
l	43.559 ± 13.71**	20.280 ± 8.83*	− 21.851 ± 14.66	− 10.295 ± 4.32*	− 6.529 ± 5.49
Epistasis	Duplicate	Duplicate	Duplicate	Duplicate	Duplicate
ICGV 06,099 × ICGV 93,468					
m	164.022 ± 2.45**	53.599 ± 4.96**	5.336 ± 9.63	21.129 ± 2.47**	33.815 ± 3.84**
d	3.825 ± 1.13**	0.508 ± 1.23	− 3.135 ± 1.93	3.891 ± 0.42**	7.431 ± 0.73**
h	− 17.694 ± 7.06*	− 30.219 ± 13.71*	77.922 ± 27.64**	21.647 ± 6.97**	17.496 ± 10.88
i	− 5.022 ± 2.45*	− 10.706 ± 4.81*	22.621 ± 9.43*	8.308 ± 2.44**	9.671 ± 3.77**
j	3.825 ± 1.13	10.085 ± 2.35	9.006 ± 4.682	− 5.963 ± 1.15	− 6.953 ± 1.80
l	12.672 ± 4.63**	22.205 ± 9.15*	− 51.588 ± 19.11**	− 14.281 ± 4.61**	− 11.036 ± 7.74
Epistasis	Duplicate	Duplicate	Duplicate	Duplicate	Duplicate

* and ** indicates significant at 5% and 1% probability levels, respectively

m = mean; *d* = additive; *h* = dominance; *i* = additive × additive; *j* = additive × dominance; *l* = dominance × dominance

found to be governing 100-kernel weight in positive direction, whereas dominance and additive × additive component were significant in negative direction. The results indicated that selection for seed weight will not be effective in early generations, since the dominant and additive × additive component have negative effect on seed size.

For pod yield per plant significant positive dominant gene action and additive × additive interaction were observed for both the crosses and significant negative dominant × dominant interaction was observed only for ICGV 06,099 × ICGV 93,468. The higher magnitude of dominance effect indicated the key role played by dominance gene effect in governing pod yield per plant as a consequence of this gene action, B_1 and F_1 recorded highest pod yield. However, the additive × additive component, which can be fixed was significant in both the crosses and it is possible to improve pod yield per plant through selection.

Gene action governing kernel Fe and Zn concentration

Significant additive gene action for kernel Fe and Zn concentrations and negative dominance × dominance epistasis for kernel Fe concentration was observed in both the crosses (Table 6). Both dominant and additive × additive epistasis was significant for kernel Fe concentration in the cross ICGV 06,099 × ICGV 93,468. The significance of dominance × dominance epistasis for kernel Fe concentration in both the crosses suggested the presence of genotypic variation and different sets of alleles in the genotypes selected for study. In breeding program, targeting enhancement of kernel Fe concentration, parental selection becomes an important criterion, wherein selection of contrasting parents is desirable. The results suggested that selection for kernel Fe and Zn concentrations in the segregating populations would be effective in the early generations.

Correlation studies

The correlation estimates of different trait-pairs are given in Table 7. Kernel Fe concentration showed highly significant positive association with kernel Zn concentration in both the crosses; 0.59 and 0.55 in the crosses, ICGV 06,040 × ICGV 87,141 and ICGV 06,099 × ICGV 93,468, respectively. Significant correlation (0.196) was also observed between 100-kernel weight and pod yield per plant for the cross ICGV 06,040 × ICGV 87,141, but it was non-significant for ICGV 06,099 × ICGV 93,468. No significant correlation was observed for 100-kernel weight and pod yield per plant with kernel Fe and Zn concentrations.

Table 7 Correlation between different pairwise traits in $F_{2:3}$ generation of crosses ICGV 06,040 × ICGV 87,141 and ICGV 06,099 × ICGV 93,468

Traits	Correlation	
	ICGV 06,040 × ICGV 87,141	ICGV 06,099 × ICGV 93,468
HKW-PY	0.196**	− 0.018 ^{ns}
HKW-Fe	− 0.225**	− 0.100 ^{ns}
HKW-Zn	0.134*	0.175**
PY-Fe	− 0.082 ^{ns}	− 0.103 ^{ns}
PY-Zn	− 0.077 ^{ns}	− 0.002 ^{ns}
Fe-Zn	0.590**	0.549**

* and ** indicates significance at 5% and 1% probability levels

^{ns} = Non-significant; HKW = 100-kernel weight (g); PY = Pod yield/plant (g/plant); Fe = Kernel iron concentration (mg/kg); Zn = Kernel zinc concentration (mg/kg)

Discussions

Studies on Fe and Zn accumulation/localization have indicated that different tissues are involved depending on the crop species (Grillet et al. 2014). In groundnut, among the kernel tissues viz., seed coat, cotyledons and embryo, cotyledons contribute more than 90% of total weight of the kernel which means that its proportional contribution to the total kernel Fe and Zn will be higher than seed coat and embryo. Thus, in case of processed/confectionery groundnut where removal of seed coat is mandatory during processing, it is still possible to retain ∼ 85–90% of total Fe and Zn in the cotyledon and embryo portion of the kernels. This is in contrast to processed cereals and millets wherein most of the Fe which was present in the aleuron layer will be lost during milling (Wang et al. 2011). Thus, groundnut based food products such as RUTFs and RUSFs developed using seeds of high Fe and Zn containing genotypes can be a suitable alternative to reducing micronutrient malnutrition largely prevalent in the developing and under developed world.

Groundnut is a self pollinated crop, therefore genetic variation governed by additive gene action and additive × additive genetic interaction effects can be exploited for trait improvement. A combination of early generation selection to tap the genetic variation governed by additive genetic effects, combined with selections in advanced generations to exploit additive × additive epistatic effects will be desirable to maximize the genetic gain for kernel Fe and Zn concentration in groundnut improvement programs. For pod yield per plant the duplicate epistasis caused a higher degree of reduction of the positive effects of dominant genes, leading to smaller yield which might be a reason for failure/difficulty in attaining higher yield in groundnut. This kind of epistasis generally hinders the improvement through selection and hence, a higher magnitude of dominance and dominance × dominance type of interaction effects would not be expected (Table 6). The role of additive × additive epistasis in governing yield was reported in groundnut (Shoba et al. 2010) and other crops that include, maize (Azizi et al. 2006), sesame (Sundari et al. 2012; Jawahar et al. 2013), and chickpea (Biranvand et al. 2013). Whereas, additive gene action governing yield per plant was reported in groundnut (Ali et al. 1999), pea (Kalia and Sood 2009), and lentil (Akbari et al. 2013). Negative significance was observed for dominance and additive × additive component of epistasis which was similar to the findings reported in of in garden pea (Singh et al. 2001); in lentil (Akbari et al. 2013) and mung bean (Noorka et al. 2014).

Thus for improving traits with high dominance or additive × additive and dominance × dominance interaction components in groundnut it is suggested to defer selection till later generations until a high level of gene fixation is attained. Also, maintenance of large populations prior to selection can be useful as it provides the maximum opportunity for advantageous combination of genes to occur. For both the crosses, the additive × dominance interaction effect was not significant for any of the studied traits.

Comparison of gene actions revealed that both additive and dominance components are important with the dominance genetic effects playing a major role for most of the studied traits viz., days to maturity, 100-kernel weight and pod yield per plant. However, in both crosses the influence of additive gene effect was noteworthy for kernel Fe and Zn concentrations. The importance of additive gene action in governing kernel Fe and Zn concentrations was also reported in other crops including common bean (Silva et al. 2013), sorghum (Gayathri 2012; Kumar et al. 2013), pearl millet (Velu et al. 2011a; Rai et al. 2012), maize (Chakraborti et al. 2011; Long et al. 2004) and rice (Zhang et al. 2004). For kernel Fe concentration, significant dominance gene effect was observed only for the cross ICGV 06,040 × ICGV 87,141, indicating the presence of different sets of alleles for this trait among the parents used in the crossing program. So, to improve the kernel Fe and Zn concentrations in groundnut selection among parental lines and pedigree method of breeding may be adopted to exploit additive component of gene effect for bringing about improvement in the target traits. Such a strategy will help in increasing the frequency of favourable alleles while maintaining a high degree of genetic variability in breeding population (Doerksen et al. 2003).

For reliable estimates of genetic effects using generation mean analysis, genes of like effects must be completely associated with the parents. Therefore, selection of parents contrasting for the trait being measured is crucial for this type of investigation. The results also indicated the important role of digenic non-allelic interactions (epistasis), among which additive × additive component was found to be influencing more number of traits especially kernel iron and zinc concentrations in the cross ICGV 06,040 × ICGV 87,141 compared to dominance × dominance component of epistasis. This assumes significance as it is very difficult to exploit the dominance component due to difficulties in developing hybrids in groundnut.

From the results obtained in the present investigation it can be concluded that there was significant influence of additive gene component for expression of kernel Fe and Zn concentrations and scope to enhance kernel Fe and Zn concentration in breeding populations through choice of parents and selections exercised in both, early and later generations. However, considering that these results are based on digenic interaction model using only two crosses, there is a scope for further study using crosses developed from contrasting parents to fit a trigenic interaction and linkage model.

Earlier studies on correlation analysis in groundnut have also showed positive association between kernel Fe and Zn concentration in selected set of germplasm and advanced breeding lines (Upadhyaya et al. 2012; Janila et al. 2014). Thus, there exists a distinct possibility of improving both traits by selecting for either of the nutrients. However, when attempting to improve both Fe and Zn concentrations in the kernels, it is suggested to base selections based on the concentration of Fe and Zn in the kernels, as independent studies on high Fe and Zn groundnut genotypes have not revealed any significant correlation between Fe and Zn concentrations (Janila et al. 2014). Similar positive association between grain Fe and Zn concentration was reported in pearl millet (Govindaraj et al. 2009; Kanatti et al. 2014), sorghum (Ravikiran et al. 2014; Susmitha and Selvi 2014), wheat (Ghanbari and Mameesh 1971; Velu et al. 2011b; Badakhshan et al. 2013) and rice (Bekele et al. 2013).

Absence of association between kernel Fe (− 0.082 ^{ns} and − 0.103 ^{ns}) and Zn (− 0.077 ^{ns} and − 0.002 ^{ns}) concentrations with pod yield per plant in both the crosses viz., ICGV 06,040 × ICGV 87,141 and ICGV 06,099 × ICGV 93,468, respectively suggested the possible simultaneous improvement of kernel iron and zinc concentration along with pod yield. Significant positive correlation of Zn concentration with pod yield with an r^2 value of 0.168 was reported earlier in groundnut (Janila et al. 2014). However, negative significant association was observed between grain Fe concentration and yield per plant (− 0.29) in pearl millet (Kanatti et al. 2014), whereas a significant positive association of grain Fe (0.374) and Zn (0.270) concentrations with yield per plant was observed in sorghum (Susmitha and Selvi 2014). In both the crosses kernel Zn concentration showed significant positive association with 100-kernel weight, however the magnitude was low.

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Declaration

Conflict of interest All the authors listed in the manuscript have agreed to the publication of the manuscript in your journal, and there is no conflict of interest.

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