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RESEARCH ARTICLE

Inheritance of factors and validation of loci linked to the kernel row number in tropical field corn (*Zea mays* L.)

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Abstract

Sustainable feeding of a growing population with nutritional security in the era of climate change is the leading challenge facing a developing nation. Field corn is one of those crops that can help achieve this goal due to its high productivity and wide adaptation. There is scope for further improving field corn productivity by targeting component traits such as kernel row number (KRN). In the present investigation, the kernel row number displayed significant variation as well asa positive correlation with yield and yield component traits under the study. The inheritance of the KRN trait was analyzed using the Wright-Castle estimator and chi-square test in two sets of F_2 populations (AH4499 and AH4500) and parental lines (AI 505, AI 541 and AI 542). The analyses by the Wright-Castle estimator revealed that KRN is governed by two effective factors (1.92@ 2) with four contributing alleles in the AH-4499 population and four effective factors (3.93 @ 4) with eight contributing alleles in the AH-4500 population. Further analysis by East's hypothesis (frequency of recessive homozygote in F_2=1/4^n) produced similar results and the Chi-square test (0.01 level of significance) confirmed the non-significant difference between expected and observed recessive frequency in F_2sof both the populations. This suggested that KRN is governed at least four genes with eight contributing alleles. In both the F_2 populations, F_1 value was non-significantly close to the mid-parent value suggesting the additive nature of KRN. Further, Bulked Segregant Analysis was carried out using AH-4500-F_2 population having 231 individuals to validate linked loci. Out of 58 flanking SSR markers previously reported for the KRN trait, only nine markers were polymorphic for this population. These linked markers identified two putative QTLs for KRN *i.e.*, *qKRN2*.1 and *qKRN2*.2 on chromosome 2 through inclusive composite interval mapping. The genetic distance with closely associated markers, bnlg 1017 was 9 cM for qKRN2.1 with a LOD score of 10.24 and a Proportion of Variance Explained (PVE%) of 16.86. The marker-trait association was further validated using $F_{2:3}$ population and it was found that the marker bnlg 1017 showed a significant association with the KRN trait. Thus, the marker bnlg 1017 could be used to identify high KRN genotypes for use in breeding programs to enhance the productivity of tropical field corn.

Keywords: Inheritance, Kernel row number, Bulked segregant analysis, Composite interval mapping, Marker validation

Introduction

Maize (*Zea mays* L.) grain yield is a cumulative effect of ear number, kernel number per ear, and kernel weight. Further, kernel number per ear can be classified into kernels per row and kernel row number (KRN) (Brown et al. 2011). Among many traits contributing to productivity, KRN is the most important trait and is positively associated with the grain yield of maize (Dhillon and Singh 1977). So, knowledge of the genetic architecture of KRN trait is required to strategically improve maize yield through trait-specific crop improvement programs. Modern many-rowed maize evolved from teosinte, a wild grass, in southwestern Mexico (Galinat 1983). Possibly, a large single mutation (Weatherwax1935), introgression (Mangelsdorf 1968), fasciations (White 1948), or a combination of these coupled with human selection led to the transformation of the two-rowed teosinte into many-rowed modern maize.

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Modern matured maize ear possesses many KRN and it is very interesting to know that at a very early stage of inflorescence development, the fate of the KRN is decided. The shoot apical meristem (SAM) is responsible for vegetative development. When the plant reaches a certain stage of growth, axillary meristem (AM) initiates the ear primordium/primordia at certain node(s) on the main stem. The terminal SAM turns into inflorescence meristem (IM) after vegetative growth stops, and further, it develops into a tassel. In maize, tassel and ear develop from similar IMs. This IM develops into a lateral meristem called a spikelet pair meristem (SPM), each SPM then, in turn, gives rise to two spikelet meristems (SM). Each SM turns into two i.e., upper and lower, floral meristems (FM) (McSteen 2006). In tassels, both upper and lower meristems develop into male flowers on the other hand, in the ear, the lower floral meristem aborts, and only the upper floral meristem develops into a kernel (Bommert et al. 2013).

The major pathway that regulates the IM's size is CLAVATA-WUSCHEL (CLV-WUS) feedback-signaling loop, which restricts stem cell proliferation by controlling the meristematic activity (Wu et al. 2018). Among several genes determining the production of large inflorescence reported so far, *fasciated ear2 (fea2)* (Bommert 1 et al. 2013a; Taguchi-shiobara et al. 2001), *thick tassel dwarf1 (td1) (*Bommert et al. 2005*),* COMPACT PLANT2 (*ct2*) (Bommert et al. 2013b) have been isolated. Other genes which regulate the size of the inflorescence meristem and cause variation in the KRN are *Corngrass1 (Cg1)* (Chuck et al. 2007), the *RAMOSA* genes (McSteen 2006), *tassels heath 4(tsh4)* (Chuck 1 et al. 2010), *unbranched2 (ub2)* and *ub3* (Chuck 2 et al. 2014), FLORICAULA*/LEAFY* (*ZFL1* and *ZFL2*). These genes influence ear architecture by regulating the SPM. Earlier, these genes were identified by genetic assays of inflorescence mutants. The hypothesis behind this is that the production of large inflorescence provides more space and, hence the development of more KRN. Many genes control the KRN and appear to show quantitative inheritance. There are ample methodologies to study the inheritance pattern of quantitative traits (Wricke and Weber 1986; Bernardo 2002). However, the expression of these many KRN genes takes place in a discrete manner and hence is considered a threshold trait (Toledo et al. 2011).

Over the years, several QTLs for the KRN have also been identified on all ten chromosomes (Cai et al. 2014). There are fewer efforts in cloning the genes associated with the KRN, especially by map-based cloning. Even though many genes involved in KRN variation have been identified *via* mutation and positional cloning, yet their genetic mechanisms are poorly understood. So, knowing the genetic basis of KRN helps the maize improvement program as KRN is one of the key yield components to be targeted for the genetic enhancement of grain yield. Besides, the dissection of the genetics of KRN also allows an understanding of maize evolution. Before going into the actual dissection of KRN genetics with the available germplasm, it is necessary to determine variability, interrelationships between traits, and linkage with markers, if any. Detailed investigation on fine mapping, sequencing, cloning, and characterization of the KRN 2 gene, indicated its negative effect on KRN in maize and it was demonstrated that knockouts of this gene on Chromosome 2 resulted in a significant gain in yield (10%) (Chen et al. 2022). Against the backdrop of the above facts, the present investigation was conducted to understand the inheritance of kernel row number, followed by identifying and validating putative makers linked to the KRN trait.

Materials and methods

Experimental materials

The materials for the experiment were chosen from the Maize Genetics Unit of ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi. The base material for the present investigation involved three fixed inbred lines, which are highly contrasting for kernel row number (KRN) trait i.e., AI 505- low KRN line (12 rows), and AI 541 and AI 542 were high KRN lines (16 and 22 rows, respectively), was selected from the pre-evaluated and characterized germplasm pool for KRN and related traits *viz*., cob length, cob girth, kernels per row and test weight (Kumar et al. 2021). Two hybrids viz., AH-4500 and AH-4499 were developed by crossing AI 505 (12 KRN) (as a common female parent) with two contrasting male parents, AI 542 (22 KRN) and AI 541 (16 KRN), respectively, during *kharif* 2019. The two hybrids were raised in separate blocks and a few F_1 individuals were self-pollinated during *rabi* 2019-20 season to obtain more than 200 F_2 seeds. A total of 231 F_2 seeds were produced in AH-4500, while AH-4499 produced 247 F_2 seeds. These seeds were grown to obtain respective F_2 populations during *kharif* 2020 season at ICAR-IARI, New Delhi.

Field experiment

The experimental location, ICAR-IARI, New Delhi is located in the northern region of India (28°08′ N latitude, 77°12′ E longitude) and receives an average annual rainfall of 797.3 mm /year during a period of 39 mean rainy days with an average temperature of 34° C. The two F_2 populations were grown in an augmented design with a 75 cm inter-row and 20 cm interplant spacing. The recommended agronomic package of practices was followed to raise a healthy crop. The individual F_2 plants were labeled to enable tracking of the candidate plants used for both genotyping and phenotyping. Phenotyping of F_2s was done for KRN and other yield-attributing traits viz., plant height, ear height, days to 50% flowering, kernel per row, ear length, ear girth,

test weight, and grain yield.

Estimation of variance for KRN trait and factors controlling it

The yield and yield component traits data were used to calculate the genetic component of variance using the formula, *Vp=Vg+Ve*. The broad-sense heritability of KRN and other yield-related traits was estimated using the formula $H^2 = (VF_2–VF_1) /VF_2$ (Burton1951). The number of effective factors or genes controlling KRN trait was estimated through "Wright -Castle" estimator using the formula, $n = (P_1–P_2)^2 /8(VF_2–V_E)$, where P_1 and P_2 represent the mean KRN of low and high KRN parents, respectively. The VF_2 is the phenotypic variance of KRN in the F_2 populations and V_E was the environmental variance. The $V_E = (VF_1+VP_1+VP_2) /3$, where VF_1 is the variance in the F_1 population, and VP_1 and VP_2 are P1 and P2 population variances, respectively (Wright1968).

Chi-square (χ2) Goodness of Fit Test

Chi-square (χ^2) goodness of fit test was performed to analyze the frequency of recessive KRN phenotype in the F_2 population using the formula, $\chi^2 = \Sigma (O - E)^2 /E$ (Pearson 1900), where O is the observed recessive KRN phenotype and E is the expected recessive KRN phenotype. The 'R-studio' statistical software with packages "Metan" and "Variability" were used for the analysis of the phenotypic data of F_2 populations.

Molecular analysis

A total of 58 SSR markers linked to KRN traits were selected based on the earlier reports (Supplementary Table S1) by considering the - Proportion of Variance Explained (PVE%) and LOD values. These markers were used for genotyping the parents, F_1 and F_2 populations.

Genotyping

DNA was extracted from fresh leaves of parents, F_1, and F_2 plants using the CTAB method (Cetyl-trimethyl ammonium bromide) as described by Sagahi-Maroof et al. 1984. The PCR reaction mixture consisting of 1X PCR buffer, 2.0 mM $MgCl_2$, 100 µM of each dNTP, 0.1 µM forward and reverse primers, and 1U *Taq* polymerase (GeneDirex) in a 25 µL volume was used to amplify the 58 selected markers. The thermal cycling profile of initial denaturation at 95°C for 5 minutes followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 52–60°C for 45 seconds to 1-minute (standardized for each primer) and extension at 72°C for 7 minutes. The PCR amplified fragments were resolved on 4% Metaphor (Lonza): Agarose (Himedia) gel in a 3:1 ratio.

Bulked segregant analysis

Bulked segregant analysis (BSA) with SSR markers was carried out to identify the marker/s linked to the KRN trait (Michelmore et al. 1991). The bulks (low KRN bulk and high KRN bulk) were generated by pooling an equal amount of DNA from the contrasting F_2 plants (ten plants each with low KRN and high KRN from the F_2 population). The identified polymorphic markers between high and low KRN parents were used to screen DNA bulks and subsequently, markers co-segregating with the KRN trait were identified and same has been validated using a set of F_2 individuals to identify the tightly linked marker in the genomic region of high and low KRN phenotype.

QTL analysis

The linkage map was constructed with the polymorphic SSR markers (Table 5) using IciMapping 4.1 software (Liet al. 2007), and the map distance between markers was calculated with the Kosambi mapping function. The QTL analysis was performed using inclusive composite interval mapping (ICIM) IciMapping software (*http://www.isbreeding.net)*. The walking speed chosen for QTL was 1cM with p = 0.001in a stepwise regression. The LOD threshold value of 3 was chosen to declare putative QTL.

Results and discussion

Kernel row number is one of the important yield component traits as well as a breeding goal for the genetic enhancement of grain yield in maize (Liu et al. 2015). KRN is conceptualized as a threshold trait and the information regarding the inheritance of threshold traits is meager, especially in plants (Toledo et al. 2011). In maize, KRN always exists in pairs. However, there is a wider variation in the expression of the trait. The genetic control of the KRN trait was earlier reported by East (1910). But the detailed studies on this trait gained momentum only during the early 21st century (Ross et al. 2006). A better understanding of the genetic architecture of KRN is required in maize breeding programs focusing on improved productivity. This investigation has been focused on understanding the genetic architecture of the KRN trait using a bi-parental population and advanced molecular tools and techniques.

Genetic variability and inheritance of KRN

Hybrid AH-4499 and its populations

The difference in the mean KRN between AI 505 (12.00) and AI 541 (16.00) was significant (at p = 0.01). The KRN varied between 10-14 rows in AI 505, whereas in AI 541 the range of KRN was 14-16 rows (Table 1). The F_1 hybrid, AH-4499, showed a mean KRN of 13.60 while the mean KRN of the F_2 population was 12.90 rows. This F_2 population recorded a genotypic variance of 28.15, a phenotypic variance of 28.76, and an environmental variance of 0.62 for the KRN trait. The heritability in broad-sense recorded by the KRN trait was 97.86. High heritability values imply that the influence of the environment is low and a high proportion of variation in a trait is due to genetic factors (Dhillon and Singh 1977). This further indicated that the KRN trait can be improved based

Table 1. Descriptive statistics, variance, and heritability of parents, F_1 and F_2 populations

Trait	Genotypes	Mean	Range	CD @ 1%	Genotypic variance	Phenotypic variance	Environmental variance	Heritability broad sense
Kernel Row Number	AI 505	12.00	10–14	1.73	28.15	28.76	0.62	97.86
	AI 541	16.00	14–16					
	AH-4499	13.60	12–14	-				
	F_2 (AH-4499)	12.90	10–16	-				
	AI 505	12.00	10–14	1.48	28.12	28.97	0.85	97.06
	AI 542	21.60	20–24					
	AH-4500	17.40	16–18	-				
	F_2 (AH-4500)	14.79	10–22	-				

on the phenotypic performance (Nzuve et al. 2014). Hence, KRN can be selected as a candidate trait for improving maize per se grain yield. The range of KRN observed in the F_2 population was 10–16 and the observed 247 F_2 individuals of the population were classified into 4 different classes of KRN, viz., 10, 12, 14, and 16 KRN with 17, 121, 98, and 11 individuals, respectively.

The output of the Wright-Castle estimator for the number of effective factors was 1.92 for KRN in the AH-4499 F_2 population (Table 2). This indicated that two genes control the KRN in AH-4499. Further, it was noticed that the frequency of recessive or lowest KRN types in F_2 was 17 out of 247. This observed F_2 frequency (17/247 ~1/16=1/4^2) was equated with the expected frequency of 1/4^n (East's hypothesis), the formula for the frequency of recessive homozygote in Mendelian genetics, where 'n' is the number of genes controlling the trait. The estimated 'n' in this case was 2. Further, the chi-square test between expected and observed recessive frequency was non-significant (0.1001). This also implied that two genes govern the KRN in AH-4499. The KRN phenotype of AH-4499-F_1 (13.6~14 rows) was almost the mid-parent value indicating the additive nature of the KRN trait. The parental lines were differing for 4 KRN and effective factors in controlling the trait were two (four alleles). This suggested that the incorporation of one contributing allele into a maize line would apparently add one extra KRN phenotype.

Hybrid AH-4500 and its populations

The difference between parental lines, AI 505 and AI 542 for KRN was significant (at $p = 0.01$) as they had 12 and 21.60, mean KRN, respectively. Since the inbred parent, AI 505 is the common female for this cross and hybrid AH-4499, the range of KRN remained the same for this parent (12-14 rows), whereas the KRN of AI 542 varied from 20-22 rows. The hybrid AH-4500 recorded a mean KRN of 17.40 and the mean KRN of the F_2 population was 14.79. The AH-4500 F2 population showed a genotypic variance of 28.12, a phenotypic variance of 28.97, and an environmental variance of 0.85 and the estimated heritability in broad-sense for the KRN trait was 97.06. Similar high h^2(b) values were observed in AH-4499 as well. The high heritability of KRN compared to

Table 2. Inheritance pattern of KRN in F_2 population of tropical maize

F_2 Population	KRN classes	Number of individuals observed	Observed recessive types	Expected recessive types	Wright-Castle estimator	Chi-square value ($p < 0.01$)
AH-4500F_2	10	1	1/231	1/256	3.93 (4)	0.0053^{NS}
	12	39				
	14	101				
	16	70				
	18	16				
	20	3				
	22	1				
AH-4499F_2	10	17	17/247	1/16	1.92 (2)	0.1001 ^{NS}
	12	121				
	14	98				
	16	11				

Table 3. Correlation among KRN and yield and yield attributing trait in population AH-4499 F_2

Traits	PH (cm)	EH (cm)	DFF	KRN	KPR	EL (cm)	EG (mm)	GY
PH (cm)	1	0.76**	-0.05	0.19**	0.10	0.26**	0.09	0.26**
EH (cm)		1	-0.15**	0.13*	-0.02	0.07	0.04	0.15**
DFF			1	-0.10	0.13*	0.16**	-0.19**	0.06
KRN				1	0.01	0.09	0.18**	0.36**
KPR					1	0.36**	-0.29**	0.57**
EL (cm)						1	0.17**	0.52**
EG (mm)							1	0.05
GY								1

Plant height (PH), Ear height (EH), days to 50% flowering (DFF), Kernel row number (KRN), kernels per row (KPR), Ear length (EL), Ear girth (EG), Grain yield (GY).

other yield component traits in maize has also been reported earlier in maize (Dhillon and Singh 1977). This means that KRN variation is mostly due to genetic causes and has a lesser influence on the environment. Hence, selection for this trait would be effective and can enhance the productivity of maize inbreds, intern enhancing the productivity of hybrids. Hence, conventional breeding procedures could lead to improvement in these traits (Lule et al. 2012). A wide range of KRN was observed in AH-4500 F_2, in which KRN ranged from 10–22 rows. Thus, 231 F_2 individuals of this population were phenotyped for KRN and they were classified into 7 different classes having 1, 39, 101, 70, 16, 3, and 1 individual, respectively with the KRN phenotype of 10, 12, 14, 16, 18, 20 and 22 rows. The Wright-Castle estimator calculated the number of effective factors in controlling KRN in the cross AH-4500 as 3.93 (Table 2), which is equivalent to four genes. It indicated that KRN is controlled by four genes in this cross. The parental lines used in this population differed for ten KRN and hence the incorporation of one favorable allele to the recessive parent will increase KRN by 1.25, which is equivalent to one KRN.

Further analysis of the frequency of recessive in the AH-4500 F_2 population revealed only one individual with extreme phenotype among 231 individuals, i.e., 1/231. This is equivalent to the expected frequency of recessive traits *i.e.*, 1/256. Using East's hypothesis, the ratio can be equated to $1/4^n$ where 'n' is the number of genes controlling the trait and accordingly, the estimated 'n' in this case was 4. The chi-square test between observed and expected frequency was non-significant (0.0053), implying the goodness of fit of East's hypothesis on the number of genes and further indicating that four effective factors are governing the KRN in AH-4500. The results from the study of the above two F_2 populations suggested that the KRN is governed, at least, by four genes (8 alleles) with a discrete variation.

In addition, in both the crosses studied, the F_1 KRN phenotype was approaching the mid-parent value, indicating the KRN trait's additive nature. It might be possible that the number of genes governing KRN is more than four. This might be understood by crossing parents with more extreme phenotypes (say KRN 8 × KRN 24), F_2 of which is expected to produce a greater number of phenotypic classes than that by the F_2s of the present study. Hence, analysis of a larger F_2 population is required to further dissect the inheritance of KRN. Thus, KRN seems to be a polygenic trait showing discrete variation and governed by additive gene action.

In the current study, in both the crosses, the parental lines used were fixed lines, and the coefficient of genetic determination (h^2) was ~1.0. Moreover, the number of rows is determined and defined ontogenetically and early. Thus, it is expected that there is no variation for KRN in the parents and F_1. However, a slight discrete variation in KRN was observed in both the parental lines and F_1 generations, while a wide variation was observed in F_2. One likely cause for this variation in parents and F_1's is that there is an environmental influence on trait expression, even though the trait is ontogenetically determined. East (1910) observed this kind of environmental influence on KRN and reported that when the upper ear is lost and there are favorable conditions, the second ear will show two additional kernel rows. It might be due to this reason that the number of individuals in different phenotypic classes of 10, 12, 14, 16, 18, 20 and 22 KRN, could not be counted accurately as some of the individuals in each class might have borne two less or two more KRN (as KRN is always an even number) and hence, the number and ratio of phenotypic classes were not as per the multiple factor hypothesis (MFH) of Nilsson-Ehle, although appeared similar to the ratio of MFH.

Correlation of KRN trait with yield and yield attributing traits

The correlation studies for the KRN trait showed a significantly positive association with plant height (0.19), ear height (0.13), ear girth (0.18), and grain yield (0.36). Similarly, the KRN trait showed a non-significant positive association with ear length (0.09) and kernels per row (0.01). In addition to KRN, plant height (0.26), ear height (0.15), number of

kernels per row (0.57), and ear length (0.52) also showed a positive significant correlation with grain yield (Table 3). It has been generally accepted that correlation between the related traits has linked physiological processes, resulting in genetic augmentation of the traits. Knowing the strength and type of association between the traits is very important and aids the breeders in initiating successful breeding programs (Breese and Haywards 1972). The results of the present study strongly suggested that genetic enhancement of KRN in field corn increases the yield potentiality of inbred lines.

Molecular analysis of KRN trait

Bulked segregant analysis for KRN trait

The BSA was carried out using parents (AI 505 and AI 542) and the F_2 population of AH-4500 (derived from the F_1 between these two parents) consisting of 231 segregants. The studied F_2 population showed normal frequency distribution for the trait (low and high KRN) (Fig. 1). Ten individuals each from high and low KRN phenotypes were selected and bulked into two groups, i.e., group 1 (low KRN) and group 2 (High KRN). These groups were subjected to molecular analysis.

A total of 58 flanking SSR markers linked to KRN trait QTLs (high LOD and PVE) were used for the polymorphic survey between the contrasting parents for trait and it was only 25 markers showed polymorphism and produced varied amplicon length, i.e., 60 to 500 bp (Table 4). This shows that there is a lot of allelic variation prevails under the studied trait. Further, each polymorphic marker used to screen bulk population (low and high) and found nine markers *viz*., bnlg1017, umc1708, umc1248, bnlg2241, umc2516, umc2027, bnlg125, bnlg339, and umc1256 were polymorphic (high variation amplicon size was represented in Table 5) between contrasting parents as well as both extreme bulk (Figs 2 and 3).

QTL analysis for KRN trait

The linkage relationship among six polymorphic markers was estimated using IciMapping software. Marker-trait-based QTL/s were identified using inclusive composite interval mapping with linked markers, viz., bnlg1017, umc1708, umc1248, bnlg2241, umc2516, umc2027, bnlg125, bnlg339, and umc1256 and it was identified two KRN QTLs on chromosome 2, i.e., qKRN2.1 and qKRN2.2, which were linked to marker bnlg1017 and umc2027, respectively (Figs. 4 and 5). The genetic distance with closely associated markers, bnlg 1017 was 9 cM for qKRN2.1 and having 10.24 and 16.86, LOD and PVE%, respectively, hence it is considered as putative QTL for the studied trait (Table 6). The flanking marker umc2027 is marked far away from qKRN2.2. Hence it is not much associated with the KRN trait. There is a huge distance between the marker and QTL. This can be narrowed down by enriching the linkage map with more markers and also developing a high-density linkage map to identify both flanking markers and establish tightly linked marker-trait association (Somssich et al. 2016). Further, the gene *KRN2* identified on chromosome number 2 (Chen et al. 2022) needs to be studied in the given population.

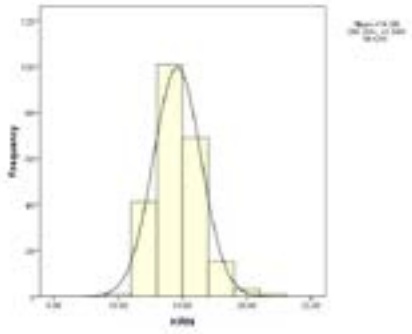

Fig. 1. Frequency distribution of KRN trait in AH-4500-F_2 population

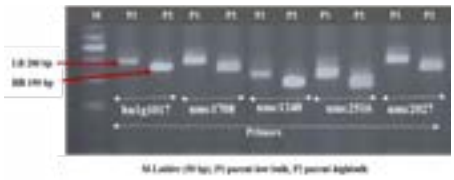

Fig. 2. Polymorphism survey using KRN QTLs linked marker

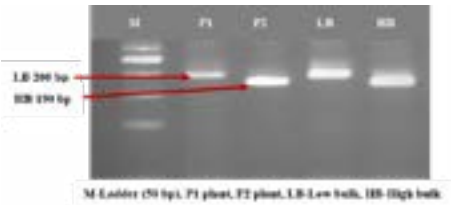

Fig. 3. Polymorphism survey (bnlg 1017) using Low Bulk and High Bulk lines

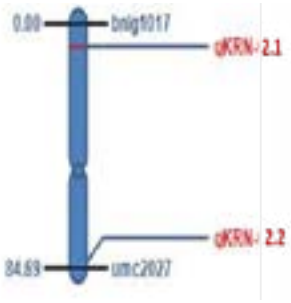

Fig. 4. Representation of QTL linked to KRN trait on chromosome 2

Validation of marker-trait linkage for KRN trait

The $F_{2:3}$ population of AH-4499, consisting of 247 individuals, was categorized based on its segregation for the KRN trait. The frequency distribution of the KRN trait showed discrete variation for KRN, indicating its oligogenic nature in AH-4499 (Fig. 6). It was noted earlier also in AH-4499by using the Wright-Castle estimator, that two genes were responsible for the KRN trait in this cross. Further, to validate the association of marker bnlg 1017 with KRN trait, 185 individuals of the

Table 4. Details of allelic variation between inbred lines (AI 505 and AI 542)

S.No.	Markers	Amplicon length (bp)	AI 505	AI 542	S.No.	Markers	Amplicon length (bp)	AI 505	AI 542
		168	+	-			325	+	-
1	bnlg1025	150	-	+	14	bnlg125	250	-	+
		250	+	-			129	+	-
2	mmc0191	140	-	+	15	umc2245	100	-	+
		138	+	-			108	+	-
3	bnlg1746	200	-	+	16	umc1012	80	-	+
		200	+	-			90	+	-
4	umc1256	128	-	+	17	umc2286	50	-	+
		250	+	-			191	+	-
5	bnlg339	134	-	+	18	umc1503	160	-	+
		150	+	-			145	+	-
6	umc1983	131	-	+	19	umc2027	155	-	+
		350	+	-			115	+	-
7	umc1708	500	-	+	20	umc1859	100	-	+
		180	+	-			240	+	-
8	umc2593	165	-	+	21	umc1248	200	-	+
		150	+	-			146	-	+
9	umc2512	144	-	+	22	umc2571	60	+	-
		160	+	-			90	+	-
10	bnlg2241	140	-	+	23	umc2093	111	-	+
		158	+	-			120	-	+
11	umc1515	110	-	+	24	umc2516	140	+	-
		133	+	-			158	-	+
12	umc1421	100	-	+	25	umc1645	150	+	-
		200	+	-					
13	bnlg1017	170	-	+					

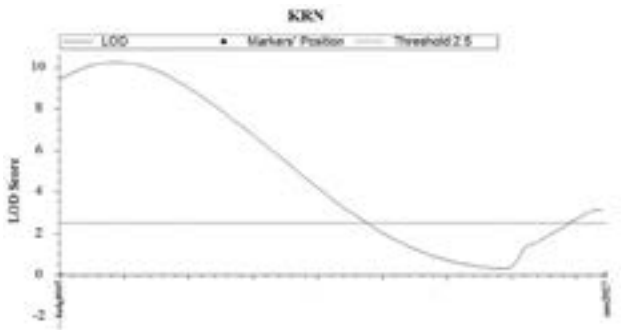

Fig. 5. Physical position of qKRN 2.1 and qKRN 2.2 on chromosome 2

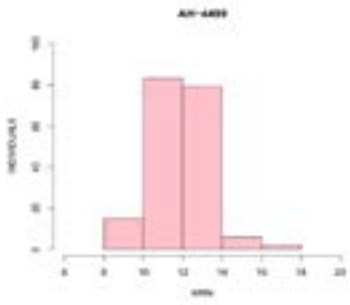

Fig. 6. Frequency distribution of KRN trait in $F_{2:3}$ population derived from AH-4499

$F_{2:3}$ populations were characterized for KRN (Fig. 7), and the segregation pattern of marker with KRN was analyzed (Supplementary Table S2).

The correlation analysis revealed a high and significant correlation (r = 0.89) between the presence of the marker and KRN phenotype. The regression analysis also indicated a significant association between KRN and marker bnlg 1017 (R^2 = 0.81). This confirmed that the bnlg 1017 linked to the KRN trait (Mukri et al. 2023). Thus, the bnlg 1017 is significantly associated with the KRN trait and explained

Table 5. Polymorphic markers identified between bulks

S. No	Markers	Amplicon length (bp)	AI 505	AI 542	Low bulk	High bulk
1	bnlg1017	200	+	-	+	-
		170	-	+	-	+
2	umc1708	350	+	-	+	-
		500	-	+	-	+
3	umc1248	240	+	-	+	-
		200	-	+	-	+
4	bnlg2241	160	+	-	+	-
		140	-	+	-	+
5	umc2516	120	+	-	+	-
		140	-	+	-	+
6	umc2027	145	+	-	+	-
		155	-	+	-	+

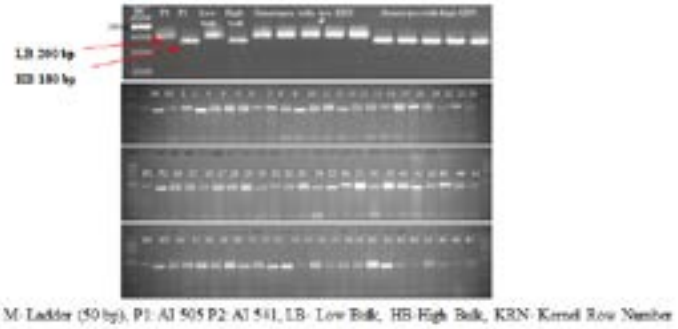

Fig. 7. Validation of linked marker bnlg 1017 on $F_{2:3}$ population segregating for KRN trait

Table 6. Details of QTL linked to KRN in maize

S. No.	Chromosome	Marker interval	Position (cM)	LoD	PVE%
1	2	bnlg 1017-umc2027	9	10.24	16.86

Logarithm of the odds (LoD), phenotypic coefficient of variation (PVE%)

16.86% of phenotypic variance, indicating that bnlg 1017 marker can be used for KRN trait improvement via marker-assisted selection field corn.

The current rate of increase of yield is hardly 0.9 to 1.3% per year for most major food crops, which is inadequate to meet the food demand of the estimated nine billion people in 2050. Hence, new and impactful component traits need to be targeted in addition to grain yield *per se* to breed for higher productivity. In the current study, a significant variation and a positive correlation was observed for the KRN trait with grain yield and with some yield components, and the inheritance pattern of KRN was understood with two populations. Further, the marker trait association study identified two QTLs i.e., *qKRN 2.1* and *qKRN2.2* located on chromosome 2 for the studied trait. Further, a saturation of markers followed by fine mapping is required to identify tightly linked maker/s that can be used in maize breeding programs for the introgression of KRN traits for improved productivity.

Supplementary materials

Supplementary Tables S1 to S2 are provided, which can be accessed online www.isgpb.org

Authors' contribution

Conceptualization of research (GM); Designing of the experiments (RNG, GM, JK); Contribution of experimental materials (GM, RNG) Execution of lab experiments and data collection (SPP, KS, CP); Analysis of data and interpretation (JSB, CS, NCG, KVG); Preparation of the manuscript (SPP, GM).

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References

Bernardo R. 2002. Breeding for quantitative traits in plants.

Woodbury: Stemma Press. **1**: p369

Bommert P., Lunde C., Nardmann J., Vollbrecht E., Running M., Jackson D., Hake S. and Werr W. 2005, thick tasseldwarf1 encodes a putative maize ortholog of the Arabidopsis CLAVATA1 leucine-rich repeat receptor like kinase. Development, **132**: 1235–45.

Bommert P., Je B.I., Goldshmidt A. and Jackson D. 2013b. The maize Gα gene COMPACT PLANT2 functions in CLAVATA signalling to control shoot meristem size. Nature, **502**(7472): 555-558.

Bommert P., Nagasawa N.S. and Jackson D., 2013a. Quantitative variation in maize kernel row number is controlled by the FASCIATED EAR2 locus. Nat. Genet., **45**(3): 334-337.

Breese E.L. and Haywards M.O. 1972. The genetic basis of present breeding methods in forage crops. Euphytica, **21**: 324-336.

Burton G.W. 1951. Quantitative inheritance in pearl millet (*Pennisetum glaucum*) Agron. J., **43**: 409–417.

Cai L., Li K., Yang X. and Li J. 2014. Identification of large-effect QTL for kernel row number has potential for maize yield improvement. Mol. Breed., **34**(3): 1087-1096.

Cai L., Li K., Yang X. and Li J. 2014. Identification of large-effect QTL for kernel row number has potential for maize yield improvement. Mol. Breed., **34**: 1087-1096.

Chuck G., Cigan A.M., Saeteurn K. and Hake S. 2007. The heterochronic maize mutant Corngrass1 results from overexpression of a tandem microRNA. Nat. Genet., **39**(4): 544-549.

Chuck G., Whipple C., Jackson D. and Hake S. 2010. The maize SBP-box transcription factor encoded by tasselsheath4 regulates bract development and the establishment of meristem boundaries. Development, **137**: 1243–1250.

Chuck G.S., Brown P.J., Meeley R. and Hake S. 2014. Maize SBP-box transcription factors unbranched2 and unbranched3 affect yield traits by regulating the rate of lateral primordia initiation. Proc Nat. Acad. Sci. USA, **111**(52): 18775–18780.

Dhillon B.S. and Singh J. 1977. Estimation and inheritance of stability parameters of grain yield in maize. The J. Agri. Sci., **88**(2): 257-265.

East E.M. 1910. A Mendelian interpretation of variation that is apparently continuous. The Am. Nat., **44**(518): 65-82.

Galinat W.C. 1983. The origin of maize as shown by key morphological traits of its ancestor, teosinte. Maydica, **28**(2): 121-138.

Han X., Qin Y., Sandrine A. M. N. and Qiu F. 2020. Fine mapping of qKRN8, a QTL for maize kernel row number, and prediction of the candidate gene. Theor. Appl. Genet., **133**(11): 3139-3150.

Kumar V.C., Gadag R.N., Mukri G., Bhat J.S., Singh C., Kumari J., Singh R.K. and Gupta N.C. 2021. Molecular characterization and multi-environmental evaluation of field corn (*Zea mays* L.) inbreds for kernel traits. Indian J. agri. Sci., **91**(11): 1622-1626.

Li H., Ye G. and Wang J. 2007. A modified algorithm for the improvement of composite interval mapping. Genetics, **175**(1): 361-374.

Liu L., Du Y., Shen X., Li M., Sun W., Huang J. and Zhang Z. 2015. KRN4 controls quantitative variation in maize kernel row number. PLoS Genet., **11**(11): e1005670.

Liu L., Du Y., Shen X., Li M., Sun W., Huang J. and Zhang Z. 2015. KRN4 controls quantitative variation in maize kernel row number. PLoS Genet., **11**(11): e1005670.

Lu M., Xie C. X., Li X. H., Hao Z. F., Li M. S., Weng J. F. and Zhang S. H. 2011. Mapping of quantitative trait loci for kernel row number in maize across seven environments. Mol. Breed., **28**: 143-152.

Lule D., Tesfaye K., Fetene M. and De Villiers S. 2012. Inheritance and association of quantitative traits in finger millet (*Eleusine coracana* subsp. *coracana*) landraces collected from eastern and south eastern Africa Int. J. Genet., **2**(2): 12-21.

Mangelsdorf P.C. 1961. Introgression in maize. Euphytica, **38**: 157-168.

McSteen P. 2006. Branching out: the ramosa pathway and the evolution of grass inflorescence morphology. The Plant Cell, **18**(3): 518-522.

Michelmore R.W., Paran I. and Kesseli R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Nat. Acad. Sci., **88**(21): 9828-9832.

Mukri G., Shilpa K., Gadag R.N., Bhat J.S., Singh C., Gupta N.C., Prabha C., Patil S.P. 2023. Designed and validated novel allele-specific primer to differentiate Kernel Row Number (KRN) in tropical field corn. PLoS ONE, **18**(4): e0284277

Nzuve F., Githiri S., Mukunya D.M. and Gethi J. 2014. Genetic variability and correlation studies of grain yield and related agronomic traits in maize. J. Agric. Sci., **6**(9): p166.

Pearson K. 1900. On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. The London, Edinburgh, and Dublin Philosophical Magazine and J. Sci., **50**(302): 157-175.

Ross A.J., Hallauer A.R. and Lee M. 2006. Genetic analysis of traits correlated with maize ear length. Maydica, **51**: 301-313.

Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A. and Allard R.W. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Nat. Aca. Sci. USA., **81**: 8014–18

Somssich M., Je B.I., Simon R. and Jackson D. 2016. CLAVATA-WUSCHEL signaling in the shoot meristem. Development, **143**(18): 3238-3248.

Taguchi-shiobara F. 2001. The fascinated ear2 gene encodes a leucine-rich repeat receptor-like protein that regulates shoot meristem proliferation in maize. Gene Dev.,**15**: 2755–2766

Toledo F.H.R.B., Ramalho M.A.P., Abreu G.B. and de Souza J.C. 2011. Inheritance of kernel row number, a multicategorical threshold trait of maize ears. *Embrapa Cocais-Artigoemperiódicoindexado (ALICE)*. Genet. Mol. Res., **10**(3): 2133-2139.

Weatherwax P. 1935. The phylogeny of *Zea mays*. The Amer. Mid. Nat. J., **16**: 1-71.

White O.E. 1948. Fasciation. The Bot. Revi., **14**(48): 319-358.

Wricke G. and Weber W.E. (Eds) 1986. Quantitative genetics and selection in plant breeding. (Berlin:deGruyter), 257-280.

Wright S. 1968. Evolution and the Genetics of Populations. I. Genetic and Biometric Foundations 1. Univ. Chicago Press, Chicago.

Wu Q., Xu F. and Jackson D. 2018. All together now, a magical mystery tour of the maize shoot meristem. Curr. Opinion Plant Biol., **45**: 26-35.

Yang J., Yeh C. T. E., Ramamurthy R. K., Qi X., Fernando R. L., Dekkers J. C. and Schnable P. S. 2018. Empirical comparisons of different statistical models to identify and validate kernel row number-associated variants from structured multi-parent mapping populations of maize. Genes, Genomes, Genet., **8**(11): 3567-3575.

Supplementary Table S1. Details of reported QTLs of KRN trait used in the study

S. No	QTL (s)	LOD	PVE (%)	Linked marker (F)	Linked marker (R)	Author (s)
1	qKRN1	4.42	2.62	umc1515	umc1323	Han et al. 2020
2	qKRN4-2	8.24	7	umc1329	phi076	Han et al. 2020
3	qKRN5	5.67	4.33	umc1852	umc2386	Han et al. 2020
4	qKRN9	4.12	4.69	umc2093	umc2338	Han et al. 2020
5	qKRN10-1	5.91	2.73	umc1293	umc1152	Han et al. 2020
6	qKRN10-2	3.65	2.93	umc2516	umc1645	Han et al. 2020
7	qKRN3	3.75	7.41	umc1012	umc1219	Han et al. 2020
8	qKRN8	7.54	8.79	umc2571	umc2593	Han et al. 2020
9	qkrn1	8.5	6.8	umc1421	umc2100	Lichuncai et al. 2014
10	qkrn3	4.8	3.9	umc2268	umc2270	Lichuncai et al. 2014
11	qkrn4	17.2	16.4	umc2027	umc1418	Lichuncai et al. 2014
12	ERN2-1	10.67	16.12	bnlg1017	bnlg125	Yang et al. 2015
13	ERN2-1	13.82	17.07	umc2245	bnlg125	Yang et al. 2015
14	ERN4-1	6.41	8.73	umc2286	umc1503	Yang et al. 2015
15	ERN4-1	12.95	12.06	umc1371	bnlg1023	Yang et al. 2015
16	ERN8-1	14.07	22.24	umc1913	umc1236	Yang et al. 2015
17	qKRN1–1	7.83	9.67	umc2396	bnlg1025	Liu et al. 2015
18	qKRN5–1	14.49	28.43	umc1365	umc1464	Liu et al. 2015
19	qKRN5–3	8.32	11.78	umc1171	bnlg1306	Liu et al. 2015
20	qKRN6–1	9.91	8.44	umc1143	umc1595	Liu et al. 2015
21	qkrn2a	11.77	10	phi092	phi127	Lu et al. 2011
22	qkrn2b	15.07	13.05	phi127	mmc0191	Lu et al. 2011
23	qkrn3a	7.63	6.14	bnlg2241	bnlg1047	Lu et al. 2011
24	qkrn7	22.2	17.86	bnlg339	umc1865	Lu et al. 2011
25	qkrn10b	13.95	10.59	phi084	phi301654	Lu et al. 2011
26	qkrn9b	8.66	7.25	phi236654	umc2089	Lu et al. 2011
27	qKRN2	10.33	14.75	bnlg1746	umc1256	Han et al. 2020
28	qKRN8	7.54	8.79	umc2571	umc2593	Han et al. 2020
29	qkrn2	16.9	16.1	umc2193	umc1259	Lichuncai et al. 2014

F = Forward primer, R = Reverse primer, QTL (s) = Quantitative trait locus, PVC = Phenotypic coefficient of variation

Supplementary Table S2. Details on the segregation pattern of $F_{2:3}$ (AH-4499) population for the marker bnlg 1017

S. No.	Progeny	Kernel Row Number	bnlg 1017 Marker score
1	1	14	-
2	2	14	-
3	3	14	-
4	4	14	-
5	5	14	-
6	6	12	-
7	7	12	-
8	8	14	+
9	9	12	-
10	10	12	-
11	11	14	-
12	12	14	-
13	13	14	-
14	14	12	+
15	15	12	-
16	16	14	-
17	17	12	-
18	18	12	-
19	19	16	-
20	20	12	+
21	21	12	-
22	23	12	-
23	24	14	-
24	25	12	-
25	26	12	-
26	27	12	-
27	28	12	-
28	29	12	-
29	30	14	+
30	31	12	+
31	32	14	-
32	33	12	-
33	34	14	-
34	35	14	-
35	36	10	-
36	37	14	-
37	38	14	-
38	39	10	+
39	40	10	-
40	41	14	-
41	42	10	+
42	43	12	-
43	44	14	-
44	45	14	-
45	46	14	-
46	47	12	+
47	48	14	-
48	49	14	-
49	50	14	-
50	52	14	+
51	53	12	-
52	54	12	-
53	55	12	-
54	56	14	+
55	57	12	-
56	58	14	-
57	59	12	-
58	60	12	+
59	61	12	-
60	63	14	-
61	64	14	-
62	65	12	-
63	66	14	-
64	67	12	-
65	68	14	+
66	69	8	-
67	70	10	-
68	71	12	-
69	73	14	+
70	74	12	-
71	75	14	-
72	76	12	-
73	77	10	+
74	78	12	-
75	79	12	-
76	80	14	-
77	82	10	+
78	83	12	-
79	84	14	-

80	85	12	+
81	87	12	+
82	88	12	-
83	91	12	-
84	92	14	+
85	93	14	+
86	94	12	-
87	96	12	-
88	97	14	-
89	98	14	+
90	99	14	-
91	100	14	+
92	101	12	-
93	102	12	+
94	103	14	-
95	104	12	+
96	107	12	-
97	108	14	-
98	109	14	-
99	110	12	+
100	111	14	-
101	113	10	+
102	116	14	-
103	117	10	+
104	118	12	+
105	119	14	-
106	120	10	-
107	121	14	-
108	124	14	-
109	125	14	-
110	126	12	+
111	127	12	+
112	128	14	-
113	129	14	-
114	130	12	-
115	131	12	-
116	132	12	+
117	133	12	-
118	134	12	+
119	135	14	-
120	136	12	+
121	137	12	-
122	138	12	+
123	139	14	-
124	140	12	+
125	141	12	-
126	142	12	-
127	143	14	-
128	144	12	+
129	145	12	-
130	146	14	+
131	147	12	-
132	148	12	+
133	149	14	-
134	150	12	+
135	152	14	-
136	153	12	+
137	154	12	-
138	155	14	+
139	157	14	-
140	158	12	-
141	159	12	-
142	161	12	-
143	162	12	-
144	163	12	-
145	164	10	-
146	166	16	-
147	167	12	+
148	168	14	-
149	169	14	+
150	170	12	-
151	171	14	+
152	172	18	-
153	173	12	-
154	174	10	-
155	175	12	-
156	176	12	-
157	177	12	+
158	178	14	+
159	179	16	-
160	180	14	-
161	181	18	-
162	182	14	-
163	183	14	+

164	187	14	-	175	200	14	-
165	188	14	+	176	201	14	-
166	191	12	-	177	203	16	+
167	192	16	+	178	204	14	-
168	193	16	-	179	205	12	+
169	194	12	+	180	211	14	+
170	195	14	-	181	212	14	-
171	196	14	+	182	213	12	-
172	197	14	-	183	214	14	-
173	198	10	-	184	215	10	+
174	199	12	-	185	216	14	-