

Analysis of maize populations for developing quality protein maize

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Abstract: Maize, being a staple food crop in a large segment of the human population, is the most suitable crop for nutritional enhancement. Quality protein maize (QPM) is a nutritionally improved commodity possessing enhanced levels of limiting amino acids such as lysine and tryptophan. In order to identify promising germplasm for developing high-yielding QPM genotypes a meta-study comprising twenty maize populations containing 441 genotypes was conducted. The experimental lines were analysed for protein quality (protein and tryptophan), and physical parameters including hundred kernel weight (HKW) and specific gravity. Twelve populations showed desirable tryptophan content ($\geq 0.51\%$). Ten populations showed high values (≥ 22) for HKW. The population S91SIWQ showed the highest value (1.289) for specific gravity. A moderately negative to strongly negative correlation has been observed between protein and tryptophan. Although a negative correlation has been observed between HKW and tryptophan content, however on the basis of data generated, three populations namely P69, S87, and S-99TLWQ-HGB were found to be the potential populations for the development of the hard kernel, high yielding QPM cultivars.

Keywords: Lysine · Maize · Protein · Tryptophan

Introduction

Cereals are the only source of nutrition for a large segment of human populations worldwide. Wheat, rice, and maize are the major cereals in Southeast Asia and Sub-Saharan Africa. Maize is known as the “Queen of cereals” due to its high yield potential and wider utilization for diverse purposes (Sethi *et al.*, 2021). It is supposed to be originated about seven thousand years ago in Central Mexico from a wild grass called teosinte. In India, it was introduced during the 17th century by the Portuguese. It is cultivated in many countries due to its wider adaptability to varied agro-climatic conditions and soil types. Generally, cereals have low protein content and as a result the populations solely dependent on cereals for deriving all of their nutritional requirements usually suffer from protein-energy malnutrition (Temba *et al.*, 2016). Although maize contributes around 15% to the global protein consumption, however, its protein quality is poor due to a higher concentration of zeins which leads to reduced levels of essential amino acids particularly lysine and tryptophan in maize kernel (Prasanna *et al.*, 2001). Zein proteins are involved in the formation of the regular protein-starch matrix which provides vitreous and hard endosperm to maize kernel. Based on their functions, zein proteins are further divided into four classes as α , β , γ and δ zeins (Wu and Messing, 2014). Zeins do not contain any lysine, with the exception of δ -zeins which contains one lysine codon (Wu *et al.*, 2012). However, many natural mutants of maize have been identified which are associated with enhanced protein quality and better kernel appearance. In 1920, a naturally-occurring mutant, *opaque-2* was identified that is associated with higher levels of essential amino acids in the maize kernel (Vasal, 2000). Since then, many more mutants like *floury-2*, *Mucronate*, *Defective*

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Endosperm 30, etc. have been identified but *opaque-2* was found to be the most suitable and thus extensively used for the development of quality protein maize. The increase in protein quality resulted from an increase in the proportion of non-zein proteins concomitant with a decrease in the proportion of zein proteins in the *opaque-2* endosperm (Jia *et al.*, 2013).

Zein proteins of maize also assume clinical significance due to their interaction with the human immune system in some patients with celiac disease. Although maize is generally considered an alternative to wheat, for such patients, however in some cases, zein proteins from maize may elicit an immune response (Cabrera-Chavez *et al.*, 2012). Hence, a reduction in the concentration of zein proteins may result in nutritionally improved maize suitable for a broader range of populations affected with nutritional disorders and celiac disease, etc.

Although the *opaque-2* gene is associated with higher lysine and tryptophan content, however, its pleiotropic effects such as soft and chalky kernel and susceptibility to insect and pest infestation, are responsible for its unpopularization (Lodha *et al.*, 2014). The intensive research interventions carried out to improve the kernel texture in *opaque-2* maize resulted in the development of present-day quality protein maize (QPM) which is characterized by a hard and vitreous kernel enriched with lysine and tryptophan content (Chaudhary, 2017). The vitreousness and hardness in the *opaque-2* kernel have been attributed to endosperm modifiers, which modify the kernel from soft and chalky to hard and vitreous. The mechanism, by which the endosperm modifiers change the grain structure from chalky to vitreous in modified *opaque-2*, is not clearly understood. The modified *opaque-2* maize with hard endosperm is known as quality protein maize (QPM). Thus, the term QPM now refers to maize homozygous for the *o2* allele, with increased lysine and tryptophan content but without the negative secondary effects of a soft and chalky endosperm (Scott *et al.*, 2004). The QPM essentially has about twice the levels of lysine and tryptophan than normal maize and also increased levels of histidine, arginine, aspartic acid, and glycine, but a reduced level of leucine (Lodha *et al.*, 2014). However, a delicate interplay is needed to be maintained between kernel modification and the protein quality threshold during the development of QPM genotypes. Complete modification of the kernel usually reduces its lysine and tryptophan below the threshold concentration required for the maize

to be termed as QPM. The QPM development, therefore, requires continuous monitoring of both protein quality and physical parameters such as hundred kernel weight and specific gravity. Further, the increasing production of maize also wants parallel improvements in its post-harvest management and therefore attention must be given to improve physical quality parameters that ensure better milling performance. Milling also determines flaking characteristics, which influence the quality of breakfast foods produced from maize.

Therefore, a need arises to evaluate a large set of maize germplasm comprising wider natural variability for protein quality characteristics and physical parameters. Keeping in view a meta-study was conducted on 441 maize genotypes belonging to twenty maize populations for protein and tryptophan content, hundred kernel weight, and specific gravity in order to identify the most suitable populations required to develop high-yielding QPM cultivars.

Materials and methods

Plant material

A set of twenty maize populations consisting of 441 genotypes received from Winter Nursery Center, Hyderabad of the Indian Institute of Maize Research was analysed for the present study. Detailed information on the experimental material is presented in Table 1. The genotypes were grown during the rainy season of 2014 in paired rows of 2 meters each. A sufficient number of ears was selfed to maintain the genetic purity of the material. The selfed ears were harvested and a sufficient quantity of kernels was shelled from the middle of the ear in order to maintain uniformity of kernel shape and size. The samples were dried in sun and processed for further analysis.

Sample screening

Seeds from selfed ears of three different replications were pooled and treated as a single accession in order to minimize the effect of biological variation of gene expression between ears. The samples were screened on lightbox to analyse the degree of opaqueness which varies from 0% to 100% with subsequent modification scores ranging from 1 to 5 i.e., 1: 0%, 2: 25%, 3: 50%, 4: 75%

Table 1. Nomenclature and correlation data of the experimental populations

S.No.	Name of population	Number of lines with protein percentage > 11 %	Number of lines with tryptophan percentage > 0.7 %	Correlation between protein and tryptophan	Correlation between tryptophan and Specific Gravity	Population size
1.	97P65	7 (1)	0	0.429	0.026	15
2.	CML 161-65	3	8	0.285	0.003	12
3.	CompMod (BC0)	0	0	0.053	0.118	14
4.	G33QC20	5	0	0.415	0.010	13
5.	P61C1	4	2	0.225	0.046	39
6.	P65C6	1	0	0.034	0.005	24
7.	P66C0	16 (4)	0	0.585	0.116	42
8.	P67C1	1	0	0.020	0.023	17
9.	P69	1	1	0.246	0.086	23
10.	S00TLYQ	2	3	0.302	0.175	23
11.	S01SIWQ	7 (3)	1	0.204	0.095	43
12.	S01SIYQ	11 (1)	0	0.426	0.017	19
13.	S87	0	0	0.234	0.353	14
14.	S91SIWQ	18 (9)	0	0.213	0.026	32
15.	S99SIYQ	0	0	0.137	0.013	10
16.	S99TLWQ-1	2	1	0.002	0.065	14
17.	S99TLWQ-HGA	0	0	0.000	0.000	14
18.	S99TLWQ-HGAB	0	3	0.001	0.187	34
19.	S99TLWQ-HGB	2	0	0.715	0.184	11
20.	S991SIWQ-ET	0	0	0.000	0.003	28

(Correlation is represented as R^2 . The number in parenthesis indicates the number of lines with a protein percentage of > 12%)

and 5: 100% opaqueness. Kernel with modification score 4, and 5 are considered *opaque-2* mutants, whereas gradation 2, and 3 is assigned to QPM. Score 1 can be considered both for QPM and normal genotypes.

Estimation of hundred kernel weight and specific gravity

Hundred kernel weight was estimated by measuring the weight of 100 maize kernels, whereas, specific gravity was analysed by measuring the rise in water volume with the addition of 10 maize kernels. For this purpose, 5 ml of water was taken in a measuring cylinder (10 ml capacity). The rise in volume was noted after the addition of 10 kernels and divided by a hundred kernel weights giving the value of specific gravity.

Sample processing

As already mentioned, equal numbers of maize kernels from 3 different ears were pooled and treated as one

sample. A minimum of three technical replicates were used for each experiment. To extract the endosperm, the kernels were soaked in water to make them soft. The germ and pericarp were then easily extracted using forceps. The endosperm was dried at low temperature in a hot air oven followed by grounding and then defatting for 36 hours using petroleum ether (40–60°C). The endosperm was dried at low temperature in a hot air oven followed by grounding in wiley mill grinder samples were stored at – 20°C for further processing.

Estimation of protein quality

Protein quality is expressed as the concentration of tryptophan in the endosperm protein. Protein content was estimated by the micro-Kjeldahl method (AOAC, 1965), whereas Tryptophan content was estimated by the papain hydrolysis method given by Hernandez and Bates (1969) and expressed as per cent tryptophan in endosperm protein. Single-step papain hydrolysis is utilized for protein

solubilisation by incubating maize samples with papain solution at 65°C overnight. Freshly prepared reagent C was used for colour generation composed of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (high purity) in glacial acetic acid and 30 N sulphuric acids. The iron ions oxidize acetic acid to glyoxylic acid in the presence of sulphuric acid. The indole ring of free tryptophan as well as that bound in soluble proteins reacts with glyoxylic acid to produce the violet-purple compound. The intensity of the violet-purple colour is measured at 545 nm. Tryptophan content was calculated with respect to the endosperm protein for each sample.

Results and discussion

As mentioned, the experimental populations were evaluated for protein, tryptophan, hundred kernel weight, and specific gravity in order to identify hard endosperm *opaque-2* lines required for breeding high-yielding QPM genotypes. Quality improvement of cereals is considered an important factor towards eradicating malnutrition prevailing in the underprivileged sections of society worldwide (Guite *et al.*, 2014). The protein content is an important quality parameter to assess the nutritional quality

of any cereal as it is a cost-effective way of protein intake. The protein content of the experimental lines is presented in Figure 1. Out of the twenty populations tested, seven populations viz. CML 161-65, 97P65, G33QC20, P65C6, P66C0, S01TLYQ, and S01SIWQ exhibited more than 9.8% of protein, the mean value averaged over all populations. The population S91SIWQ was found to possess the highest number of genotypes exhibiting more than 12% of protein. The same population also showed the highest average value for protein (11%). Two other populations viz., P66C0 and S01SIWQ contain two genotypes each with more than 12% of protein. The above populations, therefore, can be utilized for breeding high-protein maize cultivars. Protein content has been shown to be associated with dry milling characteristics as a high correlation coefficient was reported to exist between protein content and dry-milling yields of maize (Yuan and Flores, 1996). Protein content was also found to be positively correlated with the yield of flaking grits in white maize (Yuan and Flores, 1996). Maize protein also finds applications in foodstuffs for people affected by enteropathic ailments such as celiac disease. High-protein cereals are also considered to be important for healthcare

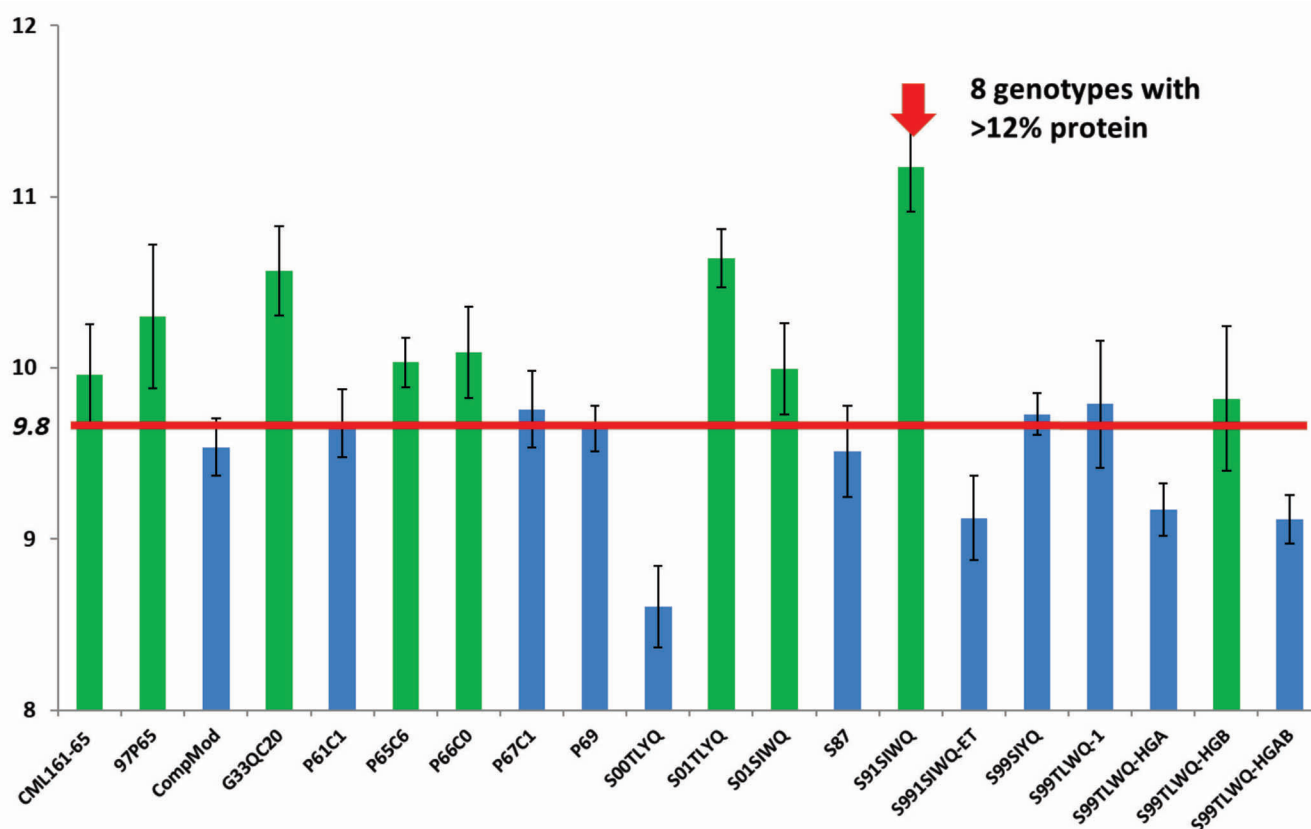


Figure 1. Protein (%) profile of the experimental populations

in patients with chronic kidney disease (Daly *et al.*, 2003). The high-protein-containing maize populations and genotypes identified in the present study can also be utilized for developing specialized maize cultivars required to produce functional foods to serve the needs of consumers with special requirements for a protein diet.

Tryptophan is one of the nine essential amino acids and a precursor for the biosynthesis of neurotransmitters (serotonin and tryptamine), vitamin (nicotinic acid), and melatonin hormone (Heine *et al.*, 1995). The tryptophan concentration of the experimental populations is presented in Figure 2. The mean value of tryptophan was found to be $0.50 \pm 0.06\%$. In normal maize, tryptophan content ranges from 0.2–0.6%, whereas it ranges from 0.5–1.1% in o2o2 maize (Vivek *et al.*, 2008). Hence, 0.51% was considered as the mean value of tryptophan to analyse diversity for protein quality in the experimental populations. Twelve population viz. CML161-69, G33QC20, P61C1, P65C6, P69, SOOTLYQ, SO1TLQ, S87, S991SIWQ-ET, S99SIYQ, S99TLWQ-1, S99TLWQ-HGAB exhibited concentration higher than the mean value of tryptophan. Population S99TLWQ-HGAB contains three, whereas the populations P61C1 and P69 contain two genotypes each

having tryptophan values of more than 0.7%. It was also observed that populations CML 161-165, G33QC20, and S99SIYQ have high amounts of both proteins as well as tryptophan. Hence, these lines can be used for breeding high-protein QPM cultivars. The higher tryptophan concentrations ($\geq 0.6\%$) observed in sweet corn populations (S00TLQ, S87, and S99SIYQ) might be the result of the shrivelled grain size usually observed in sweet corn kernels.

A highly positive correlation has been reported to exist between tryptophan and lysine content in maize endosperm and an increase in the percentage of tryptophan is accompanied by a concomitant increase in lysine (Vivek *et al.*, 2008). Due to ease of its quantification, tryptophan content is usually analysed in order to quantify the protein quality in maize. The amount of tryptophan and lysine was found to be double in QPM as compared to normal maize (Vivek *et al.*, 2008). The health benefits of QPM consumption are well-documented. QPM has been instrumental in fulfilling the protein needs in low-resource settings of the world (Nuss *et al.*, 2011).

Hundred kernel weight (HKW) is an important trait in selecting promising lines for the development of hard

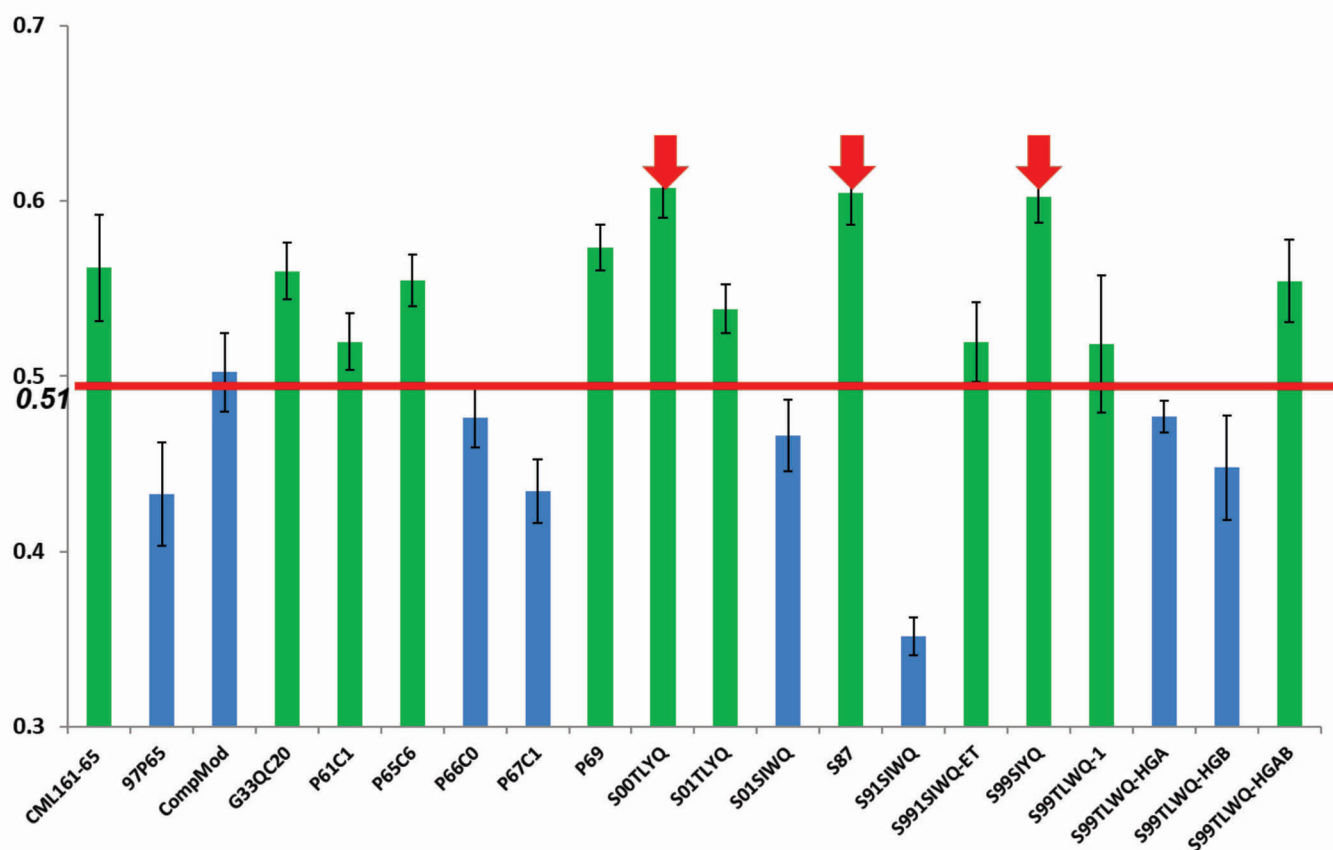


Figure 2. Tryptophan content (%) of the experimental populations

kernel QPM genotypes. The distribution of HKW amongst 20 experimental populations is presented in Figure 3. The mean value of HKW was found to be 22.0 ± 4 . Ten populations were found to possess HKW higher than the mean value. Population S91SIWQ exhibited the highest values for HKW (29.52), whereas G33QC20 exhibited the lowest (18.3) HKW. Hundred kernel weight is an important parameter contributing toward higher grain yield. It is associated with kernel number, a trait which is further correlated with ear length, ear row number, and kernel number per row (Chen *et al.*, 2016). Much work is currently being done to elucidate putative QTLs for this trait. Kernel weight also determines seed size for optimal crop density and is also used for calibrating seed drills and for estimating shattering losses during harvest. HKW has also been found to positively influence the test weight of maize, a parameter important to the yield of large flaking grits (Paulsen and Hill, 1985).

Specific gravity is also an important quality trait as along with porosity, it determines kernel hardness, susceptibility to breakage, rate of drying, and propensity to disease development (Chang, 1987). The mean value of specific gravity in the experimental populations was

found to be 1.2 ± 0.5 (Figure 4). Eight populations showed specific gravity higher than (1.2). The population S91SIWQ showed the highest value (1.289), whereas S87 exhibited the lowest (1.129). Maize grains are reported to have a high porosity of around 12–13%. (Chang, 1987). The nutritional composition of the kernel including starch, amylose/amylopectin ratio, protein, and oil, and its packaging, influences kernel density (Paulsen *et al.*, 2003). While hard kernel maize is desired for the production of dry milled products like flour and grits, soft kernels find applications in feed, ethanol, and glucose/fructose production as less steeping time is required for maize having soft kernels. Kernel hardness, therefore, needs to be optimized as per the end-use application.

HKW and specific gravity are important traits in developing QPM genotypes as QPM is defined as the maize homozygous for the *o2* allele with increased lysine and tryptophan content along with hard and vitreous endosperm. The high tryptophan *opaque-2* genotypes developed during the 70's and 80's remained unpopular due to soft and opaque kernel which leads to lower yields and susceptibility to insects and pest infestation. The development of hard kernel genotypes becomes a necessity in order to harness the

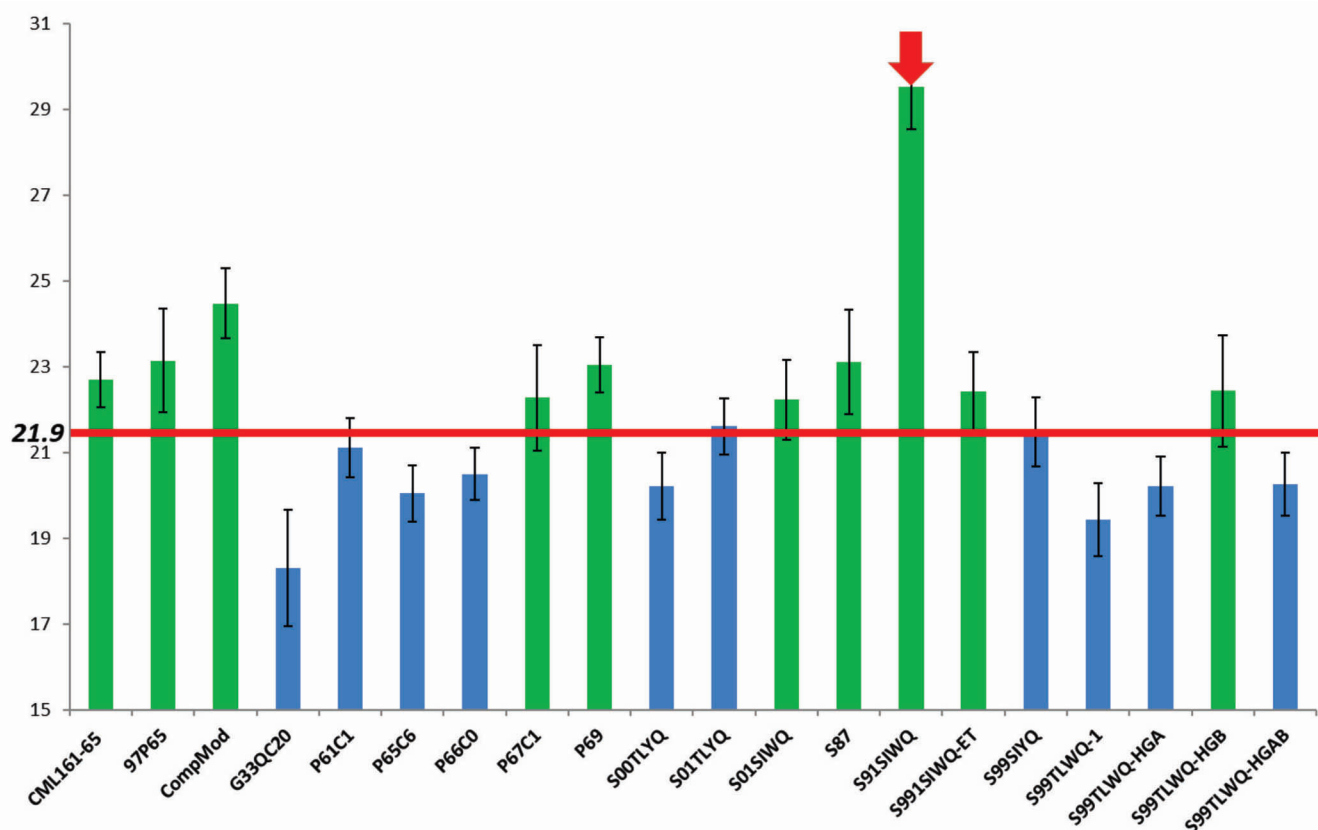


Figure 3. Hundred kernel weight of the experimental populations

benefits of nutritionally improved *opaque-2* maize. Since specific gravity is directly associated with the kernel hardness, therefore, the estimation of lysine and tryptophan along with 100 kernel weight and specific gravity measurement is a must for developing hard kernel high yielding QPM cultivars. Recently, fine-mapping of a QTL region *qGW4.05* has revealed a candidate gene responsible for the HKW trait (Cheng *et al.*, 2016). The molecular information can further be utilized to improve the character of elite maize lines.

From the results, it is observed that populations CML 161-165, P69, and S01TLYQ have a high content of protein, tryptophan, and HKW traits. S91SIWQ contains higher amounts of protein along with high HKW and specific gravity. None of the experimental populations showed high values for all four traits. However, it is observed that populations *viz.*, CompMod and S991SIWQ-ET may be used to breed high-yielding QPM cultivars as they possess high value for HKW and specific gravity along with higher amounts of tryptophan. It is also found that tryptophan content showed a moderately negative correlation with specific gravity. This is expected since a lesser amount of prolamin fraction of proteins in *opaque-*

2 maize results in soft and opaque kernels owing to the incomplete starch-protein matrix. The kernel hardness and specific gravity are, therefore, important traits in developing hard endosperm QPM genotypes.

The data on the correlation between various traits are presented in Tables 1 and 2. Although no strong correlation has been observed among any of the parameters, however, the population-wise data analysis revealed that five out of the twenty populations showed a moderate to strong negative correlation between tryptophan and protein contents. Hence, it can be concluded that the negative correlation usually observed between protein and tryptophan is not universal and presumable depending on gene action. Interestingly, in one population S99TLWQ-HGB, a strong positive correlation is found between protein and tryptophan contents. This population can be assessed for gene action and the development of new germplasm/breeding lines. Although a negative correlation has been observed between HKW and tryptophan content, however, on the basis of data the populations P69, S87 and S-99TLWQ-HGB can be utilized for the development of the hard kernel, high-yielding QPM cultivars.

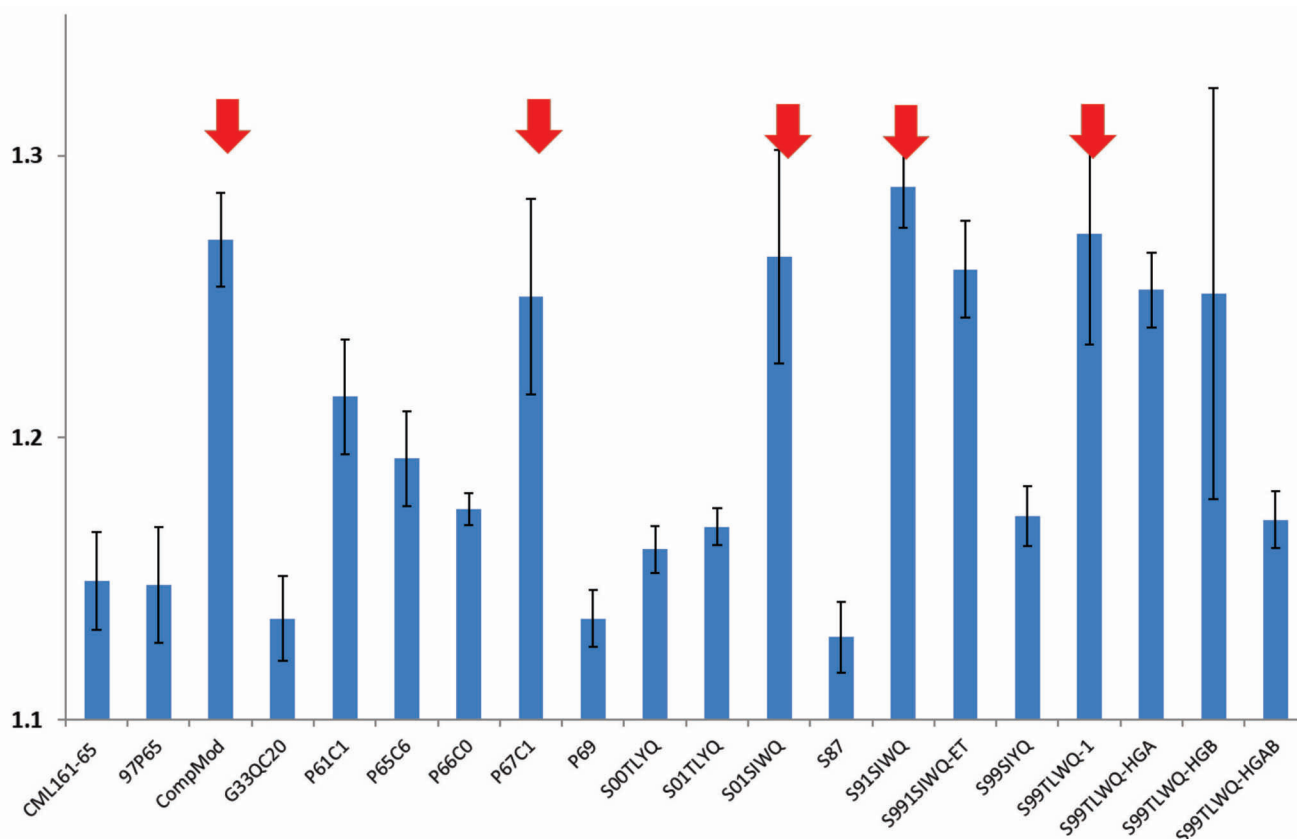


Figure 4. The specific gravity of the experimental populations

Table 2. Populations showing a moderate to strong correlation between protein and tryptophan contents

S.No.	Name of the population	Pearson correlation coefficient (R)	Coefficient of determination (R ²)	Nature of correlation	Significance level
1.	97P65	- 0.6654	0.429	Moderate negative	0.05
2.	G33QC20	- 0.6442	0.415	Moderate negative	0.05
3.	P66C0	- 0.765	0.585	Strong negative	0.01
3.	S01SIYQ	- 0.653	0.426	Strong negative	0.01
4.	S99TLWQ-HGB	0.8457	0.715	Strong positive	0.01

Conclusion

The present study immensely helped to identify potent maize populations required for breeding high-yielding hard kernel QPM genotypes. The data on correlations amongst different quality parameters in maize might be helpful for designing the future breeding program for the development of nutritionally improved maize. Opaqueness, related to tryptophan content, results in higher porosity due to the enlargement of starch granules and reduction in the size of protein bodies. The increased porosity may be expected to result in a decrease in specific gravity. However, analysis in the present study does not reveal any correlation between specific gravity and tryptophan content, demonstrating that other mechanisms may be in place apart from the reduction in the size of protein bodies. The present study clearly elucidated some potential populations (CML 161-165, P69, S01TLYQ, S91SIWQ) with desired physical parameters like hundred kernel weight and specific gravity along with protein and tryptophan which can effectively be utilized in the development of high-yielding, hard kernel QPM genotypes.

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References

- AOAC. (1975). Association of Official Agricultural Chemists, official methods of analysis, Washington, D.C.
- Cabrera-Chavez, F., Iametti, S., Miriani, M., de la Barca, A. M., Mamone, G. & Bonomi, F. (2012). Maize prolamins resistant to peptic-tryptic digestion maintain immune-recognition by IgA from some celiac disease patients. *Plant Foods for Human Nutrition*, **67**: 24–30.
- Chang, C. S. (1987). Measuring density and porosity of grain kernels using gas pycnometer. *Cereal Chemistry*, **65**: 13–15.
- Chaudhary, D. P., Kumar, S. & Singh, S. (2014). Maize: Nutrition dynamics and novel uses. Springer India.
- Chen, J., Zhang, L., Liu, S., Li, Z., Huang, R., Li & Ding, J. (2016). The genetic basis of natural variation in kernel size and related traits using a four-way cross population in maize. *PLoS ONE*, **11**: 1–12.
- Chen, L., Li, Y. X., Li, C., Wu, X., Qin, W., Li, X., Jiao, F., Zhang, X., Zhang, D., Shi, Y., Song, Y., Li, Y. & Wang, T. (2016). Fine-mapping of qGW4.05, a major QTL for kernel weight and size in maize. *BMC Plant Biology*, **16**: 81–94.
- Daly, A., Franz, M. & Holzmeister, L. A. (2003). Exchange lists for meal planning. *American Diabetes Association, Alexandria*, **9**: 10–15.
- Guite, N., Ghosh, S. & Brahmachari, A. (2014). Addressing issues of malnutrition in children through public nutrition using local resources of agriculture and land use: Evidence from the field based evaluation study in Uttar Pradesh. *Indian Journal of Community Health*, **26**: 237–244.
- Heine, W., Radke, M. & Wutzke, K. D. (1995). The significance of tryptophan in human nutrition. *Amino Acids*, **3**: 91–205.
- Hernandez, H. & Bates, L. S. (1969). A modified method for rapid tryptophan analysis of maize. Research Bulletin 19th edn, pp. 1–42. International Maize and Wheat improvement centre, Mexico.
- Jia, M., Wu, H., Clay, K. L., Jung, R., Larkins, B. A. & Gibbon, B. C. (2013). Identification and characterization of lysine-rich proteins and starch biosynthesis genes. In the opaque2 mutant by transcriptional and proteomic analysis. *BMC Plant Biology*, **13**: 60–69.
- Nuss, E. T. & Tanumihardjo, S. A. (2011). Quality protein maize for Africa. Closing the protein inadequacy gap in vulnerable populations. *Advances in Nutrition*, **3**: 217–224.
- Paulsen, M. R., Watson, S. A. & Singh, M. (2003). Measurement and maintenance of corn quality. *Corn: Chemical Technology*, **2**: 159–220.
- Prasanna, B. M., Vasal, S. K., Kassahun, B. & Singh, N. N. (2001). Quality protein maize. *Current Science*, **81**: 1308–1319.
- Rosenzweig, C., Tubiello, F. N., Goldberg, R., Mills, E. & Bloomfield, J. (2002). Increased crop damage in the US from excess precipitation under climate change. *Global Environmental Change*, **12**(3): 197–202.
- Scott, M. P., Bhatnagar, S. & Betran, J. (2004). Tryptophan and methionine levels in quality protein maize breeding germplasm. *Maydica*, **49**: 303–311.
- Sethi, M., Singh, A., Kaur, H., Kumar, R., Rakshit, S. & Chaudhary, D. P. (2021). Expression profile of protein fractions in the

- developing kernel of normal, opaque-2 and quality protein maize. *Scientific Reports*, **11**: 2469.
- Temba, M. C., Njobeh, P. B., Adebo, O. A., Olugbile, A. O. & Kayitesi E. (2016). The role of compositing cereals with legumes to alleviate protein energy malnutrition in Africa. *International Journal of Food Science and Technology*, **51**(3): 543–554.
- Vasal, S. K. (2000). The quality protein maize story. *Food and Nutrition*, **21**: 445–450.
- Vivek, B. S., Krivanek, A. F., Palacios-Rojas, N., Twumasi-Afriyie, S. & Diallo, A. O. (2008). Breeding Quality Protein Maize (QPM): Protocols for developing QPM cultivars. Mexico, CIMMYT.
- Wu, Y. & Messing, J. (2014). Proteome balancing of the maize seed for higher nutritional value. *Frontiers in Plant Science*, **5**: 240–254.
- Wu, Y., Wang, W. & Messing, J. (2012). Balancing of sulphur storage in maize seed. *BMC Plant Biology*, **12**: 77–87.
- Yuan, J. & Flores, R. A. (1996). Laboratory dry-milling performance of white corn: effect of physical and chemical corn characteristics. *Cereal Chemistry*, **73**: 574–578.