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Research Article

Evaluation of pro-vitamin A enriched maize hybrids for fighting hidden hunger in Nepal

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Abstract

Prevailing vitamin A deficiency is a malnutrition repercussing retarded growth, weak immune system and night-blindness in human beings. Pro-vitamin A enriched maize hybrids could be a strategy for combating vitamin A deficiency, mostly prevailing in children and women of Nepal. With the objective to investigate superior pro-vitamin A enriched 'bio-fortified' maize cultivars, twice replicated experiments were laid out in α-lattice design over two consecutive growing seasons of 2019 and 2019/20 at the National Maize Research Program (NMRP), Rampur, Chitwan, Nepal. The results revealed that the difference among tested hybrids was glaring for all agro-morphological, yield, and yield components traits. Among the evaluated traits, days to 50% anthesis and silking, plant and ear height, numbers of kernel rows per cob, grains per row, and grain yield varied significantly among the tested maize hybrids. Effect of planting season was significant for grain yield where winter maize produced 32% higher grain yield than spring maize. HPO16-2, HPO49-3, HPO49-5, and HPO49-2 were the 38-61% high yielding 'bio-fortified' maize genotypes than normal hybrid check. Therefore, these hybrids might be the potential higher-yielding future pro-vitamin A enriched maize hybrids to resolve food insecurity, malnutrition, trade deficit on maize grains and specially to combat vitamin A deficiency in Nepal.

Introduction

Maize is serving as an important staple crop in Nepal. It can play a vital role in ensuring food security due to its high productivity and commercial versatility. It is produced and consumed as a main food in the areas where food and nutrition insecurities are the major challenges. The crop is also used as major feed ingredient for commercial poultry, livestock, and goat in hills as well. Hence, maize is a dual-purpose crop widely used as a food for humans and feed for livestock. The present demand for maize grains has been extensively enhanced on account of their use in poultry and livestock feed. However, the local production of maize does not meet the national feed demand due to

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fast-growing poultry and dairy farming (Osti, 2019). It can be cultivated in all three crop seasons, across all ecological regions, and fits well into crop rotations either as a single or in rotation/mixed/multi-cropping. It is gaining an important position in the cropping system due to higher yield potential, short growing period, higher food value and multiples uses (Taipodia & Shukla, 2013). However, the challenge is the large and growing food security gap in many places around the country due to ever increasing population. In general, yellow maize hybrids produced in winter at Terai is mostly used for feed while white/yellow maize of hills sown in spring or summer is used for food as well as feed. Yellow colored maize is commonly used for feed and is important for 25% of farmers who are doing commercial farming while white/yellow-colored maize is consumed as human food by the remaining 75% of subsistence farmers (Simkhada, 2020). Nearly 80% of the production in Terai is sold to poultry and animal mills, and 86% of maize produced in the hills is used at the household level (Thapa et al., 2021). Normal white maize that dominates the maize food markets can provide sufficient quantities of energy to the human diet, but do not contain significant amounts of micronutrients. Bio-fortification could be most impactful, convenient, suitable and acceptable intervention to overcome malnutrition (Maqbool & Beshir, 2019).

The prevalence of 'hidden hunger' due to micronutrient deficiency is common problems among the rural poor where maize is consumed as staple food (Bhandari & Banjara, 2015). A healthy diet is considered to be one that satisfies human needs for energy and all essential nutrients (FAO 2004). Our national food system is falling to deliver adequate quantities of healthy, nutritionally balanced food, especially to the resource poor underprivileged people leading to micronutrient malnutrition. Therefore, food as well as nutrition security is one of the major challenges for humanity. Minerals (Fe, Zn) and Vitamin A malnutrition are major food related public health problem among children and women of Nepal (Fiedler, 2000) where large fraction of population depended on cereal-based diet, and limited access to meat, fruits, and vegetables. Vitamin A deficiency causes night blindness, growth retardation, xerophthalmia and increases the susceptibility against epidemic diseases (Maqbool et al., 2018). Pro-vitamin A (PVA) enriched maize is a special type of bio-fortified 'orange' maize that contains high levels of β-carotene. Yellow and orange maize can naturally produce vitamin A carotenoids, including α-carotene, β-carotene, and β-cryptoxanthin, which can be metabolically converted to active vitamin A in the human body (Asson-Batres & Rochette-Egly, 2016). However, kernels of yellow maize cultivars commonly grown by farmers contain less than 2 μg g^{-1} of PVA (Ortiz-Monasterio et al., 2007; Pixley et al., 2013), which is insufficient to meet the recommended daily requirement. Various efforts have thus been made to increase the concentrations of PVA carotenoids in maize through conventional and molecular marker-assisted breeding (Pixley et al., 2013; Giuliano, 2017; Andersson et al., 2017). Study conducted by Muzhingi et. al (2017) shows that, biofortifed maize for pro vitamin A contains zeaxanthin 1.2–13.2 μg g^{-1} dry weight, β-cryptoxanthin (1.3–8.8 μg g^{-1} dry weight) and β-carotene (1.3–8.0 μg g^{-1} dry weight). In addition, vitamin E compounds identified in provitamin A carotenoids biofortified maize are α-tocopherol (3.4–34.3 μg g^{-1} dry weight), γ-tocopherol (5.9–54.4 μg g^{-1} dry weight), α-tocotrienol (2.6–19.5 μg g^{-1} dry wieght) and γ-tocotrienol (45.4 μg g^{-1} dry weight). Also, The ranges of phenolic compounds are γ-oryzanol (0.0–0.8 mg g−1 DW), ferulic acid (0.4–3.6 mg g^{-1} dry weight) and p-coumaric acid (0.1–0.45 mg g^{-1} dry weight).

High yielding bio-fortified "orange" maize hybrids can provide double benefits that can benefits both commercial as well as subsistence farmers. Improving the nutritional status of children and adults is a highly effective way to increase economic productivity in agriculture and other sectors. Linking agriculture and nutrition to promote dietary change and improve nutritional status can generate wide economic benefits, such as increased agricultural production and greater household security. It could be the three problems solving solution to address food insecurity, malnutrition and trade deficit of maize grains by single capsule. Hence, this can significantly reduce the prevalence of "hidden hunger" due to micronutrient deficiency, food shortage, and decrease import of maize grains which can ultimately make difference in the well-being of people. It is imperative for National Maize Research Program to identify and develop high yielding PVA enriched bio-fortified maize hybrids. Furthermore, it is necessary to teach farmers who are not aware of health benefits of 'orange' maize for ensuring adoption of bio-fortified varieties. Increasing maize production by using bio-fortified maize can be considered as one of the most important strategies to curb food and nutritional insecurity in the country. This is because maize; (i) is widely produced and consumed in the country (ii) has higher yield and commercial potential and (iii) fits well into crop rotation than other crops. The main objective of the PVA enrichment with higher yield in maize breeding program has been to develop high-yielding, provitamin A-enriched maize cultivars which can be profitable and acceptable to the farmers comparing with other local and improved varieties along with proven effectiveness in reducing vitamin A deficiency (Bouis & Welch, 2010). Therefore, the current study was designed to identify high yielding PVA enriched bio-fortified 'orange' maize hybrids suitable for Nepal. The objective of this study was to investigate superior pro-vitamin A enriched 'bio-fortified' maize cultivars out of twenty-nine hybrids received from international trials of CIMMYT, Mexico. Identifying the superior pro-vitamin A enriched hybrids, which will have high potential to grow in Terai and Inner Terai location of Nepal, opens the door to all the hybrid seed stakeholders to reach out to farmers and consumers, eventually, contributing to combat hidden hunger of Nepal.

Materials and methods

Location and plant materials

This study was carried out at National Maize Research Program (NMRP), Rampur, Nepal (27°40'N latitude and 84°21'E longitude with an altitude of 228 meters above sea level) in the spring of 2019 and winter of 2019/20. Twenty-nine Provitamin A (PVA) enriched "orange" maize genotypes developed by CIMMYT, Mexico and one check variety, Rampur Hybrid-10 (RH-10), were used in this study (Table 1). This experimental set was received from Nepal Seed and Fertilizer Project (NSAF/CIMMYT) funded by USAID. Rampur Hybrid-10 was used as a local check-in both years.

Table 1. List of genotypes used for research

S.N	*Genotype*	*Source*	*S.N*	*Genotype*	*Source*
1.	HP049-24	CIMMYT, Mexico	16.	HP049-19	CIMMYT, Mexico
2.	HP049-9	CIMMYT, Mexico	17.	HP049-17	CIMMYT, Mexico
3.	HP049-18	CIMMYT, Mexico	18.	HP049-21	CIMMYT, Mexico
4.	HP049-2	CIMMYT, Mexico	19.	HP049-8	CIMMYT, Mexico
5.	HP049-11	CIMMYT, Mexico	20.	HP049-4	CIMMYT, Mexico
6.	HP049-10	CIMMYT, Mexico	21.	HP049-25	CIMMYT, Mexico
7.	HP049-3	CIMMYT, Mexico	22.	HP049-26	CIMMYT, Mexico
8.	HP049-1	CIMMYT, Mexico	23.	HP049-15	CIMMYT, Mexico
9.	HP049-13	CIMMYT, Mexico	24.	HP049-27	CIMMYT, Mexico
10.	HP049-22	CIMMYT, Mexico	25.	HP1134-6	CIMMYT, Mexico
11.	HP049-7	CIMMYT, Mexico	26.	HP049-16	CIMMYT, Mexico
12.	HP049-5	CIMMYT, Mexico	27.	HP049-20	CIMMYT, Mexico
13.	HP049-14	CIMMYT, Mexico	28.	HP049-23	CIMMYT, Mexico
14.	HP049-12	CIMMYT, Mexico	29.	HP049-6	CIMMYT, Mexico
15.	HP016-2	CIMMYT, Mexico	30.	RH-10	NMRP, Rampur

Experimental details

In both years, the maize genotypes were arranged in α-lattice design with two replicates. Each plot consisted of two 4-m long row ridges with row spacing of 0.75-m and plant spacing of 0.20-m within a row. The recommended rate of fertilizer was applied (180:60:40 kg ha^{-1} N: P2O5:K2O) in the form of Urea, Di-ammonium phosphate, and Murate of Potash. A full dose of Phosphorous and Potash along with farm yard manure (15 t ha^{-1}) was applied as basal application. Urea was top-dressed as three split doses. Immediately after sowing, Attrazin @ 2.0 g + Pendimethalin @ 4.5 ml L^{-1} was applied to control pre-emergence weeds. Plots were kept free of weeds during the crop season by two manual weeding and inter cultural operations. Irrigation was provided six to eight times during the entire crop cycle as needed.

Data recording and analysis

The following traits were measured at the flowering and/or post-flowering stage. Days to 50% anthesis (AD), days to 50% silking (SD), plant height, ear height, and cob length were measured as the method suggested by International Maize and Wheat Improvement Centre (CIMMYT). Numbers of plants, numbers of ears, and field weight were recorded on plot basis. The recorded per plot field weight (kg) was converted into grain yield (t ha^{-1}) by multiplying the conversion factor 0.8 with a 12.5 percent moisture adjustment. Analysis of variance of α-lattice design for single-season and combined data was performed based on individual plot observation using META-R software developed by CIMMYT (Alvarado et al., 2015).

Results and discussion

It is imperative to evaluate and find appropriate bio-fortified maize hybrids suitable for spring and winter planting in Nepal. The sowing time gives higher differences in grain yield because the performance of maize usually depends on photoperiod. The twenty-nine PVA enriched hybrids presented significant differences in all traits under study except numbers of plants and ears. There were considerable differences in tested hybrids in different seasons even within the same variety. From the results, it is clear that some hybrids achieved excellent performance in both seasons probably due to the inherent genetic variation.

Agro-morphological and phenological traits

Days to anthesis and silking are important reproductive traits usually utilized by plant breeder as the basis for deciding the maturity group of the cultivars. High heritability (>0.80) calculated for days to 50% anthesis and silking in both the years (Table 1). The duration of anthesis and silking differed according to the season. In this study, days to 50% anthesis and silking were around two times lengthy in winter than spring planting. Differences recorded between the genotypes for days to 50% anthesis and silking but non-significant differences were observed in genotype × year interaction just for anthesis (Table 2). In this study, reproductive traits have higher heritability than vegetative traits. A similar conclusion was reached by Buso, Gomes, Ballesta, and Mora (2019). Anthesis and silking interval is important for synchrony between male and female flowers. Analysis of variance for days to anthesis and silking revealed highly significant differences among the genotypes. This result ties well with previous studies of Khan et al. (2018) wherein highly significant differences among the genotypes for these traits were due to the presence of genetic variability in the studied materials. The duration of flowering was highly different between the spring and winter seasons but the amount of heat required to complete the flowering stage does not vary. The findings are directly in line with previous study of Parthasarathi, Velu, and Jeyakumar (2013) wherein the combination of temperature and time i.e. growing degree day or measure of heat accumulation is always be the same in maize irrespective of growing seasons. This is also consistent with what has been found in previous study of Nielsen et al. (2002).

The observed heritability for plant height was low in spring and high in winter whereas it was medium in spring and high in winter for ear height (Table 1). In contrast to flowering duration, both plant and ear height was taller in spring as compared to winter planting. Genotypic differences observed for both plant and ear height. Plant height was recorded nearly 30% taller and ear height was nearly 50% more in spring. The ear placement in the population was exactly half of plant height in spring and 43.5% of plant height in winter (Table 1). The placement of ear in Rampur Hybrid-10 (RH-10) was exactly on middle of the plant (Table 2) while top yielding genotypes had nearly ≥45% and low yielding genotypes had nearly 40% of plant height. Plant and ear height are important traits not only for describing new varieties but also for green and dry matter production, determination of stalk lodging, and even for grain yield in maize as described by Li, Dong, Niu, & Cui (2007) and Zsubori et al. (2002). The plant height of different varieties of maize does not remain equal because of differences in the genetic makeup of the genotypes. The growing season has a substantial effect on plant and ear height, and the crop responded absolutely to planting time. This result is following the previous results of Ali, Ali, Ahmad, Igbal, and Anwar (2018) who reported a difference of plant height in different maize varieties. The crop was highly sensitive to temperature and the winter crop was always shorter than spring crop. The results lead to a similar conclusion with Zaidi et al. (2010) who explained that crop growth rate was reduced while growth duration was prolonged due to low temperature. In winter maize, cold during the early stages of development alters plant phenology because of its negative impact on photosynthesis as suggested by Revilla, Malvar, Cartea, Butrón, and Ordás (2000).

Table 2. Descriptive statistics of pro-vitamin A enriched maize hybrids for the characters recorded on two consecutive years in Rampur, Chitwan

Characters	2019 (spring)				2019-20 (winter)			
	h2	Range	Mean±SEM	Sig.	*h2*	Range	Mean±SEM	Sig.
Days to 50% anthesis	0.85	48-56	52±0.2	**	0.83	98-113	105±0.4	**
Days to 50% silking	0.88	49-57	53±0.3	**	0.85	99-113	107±0.4	**
Plant height, cm	0.12	203-260	236±1.8	ns	0.71	130-225	184±2.2	*
Ear height, cm	0.55	83-148	118±1.8	ns	0.68	40-115	80±2.0	*
No. of plants ha^{-1}	0.17	26667-55000	40417±774	ns	0.00	26667-63333	46556±1199	ns
No. of ears ha^{-1}	0.32	28333-53333	40528±733	ns	0.24	30000-116667	66500±2112	ns
Cob length, cm	0.77	15.4-20.8	18.6±0.15	**	0.76	13.6-19.5	16.4±0.19	**
No. of kernel rows cob^{-1}	0.76	12.4-16.8	13.8±0.13	**	0.64	11.3-16.7	13.8±0.13	**
No. of grains row^{-1}	0.55	28.8-42.8	36.9±0.36	*	0.46	21.3-32.7	27.6±0.35	ns
Grain yield (t ha^{-1})	0.48	3.47-8.47	5.23±1.23	*	0.46	2.98-10.59	6.92±2.42	*

Sig.=significance, * significant at ≤0.05 p level,** significant at ≤0.01 p level, and ns=non-significant at 0.05 p level

Yield and yield component traits

The numbers of plants and ears had low heritability. Ear to plant ratio of 1.0 and 1.40 was recorded respectively in the experiment of 2019 spring and 2019/20 winter (Table 1). High heritability for cob length and medium heritability for numbers of grains per row was recorded. In general, it seems that plants having single ear had long cob and more numbers of grains per row than double ears. High heritability with exactly the same value was demonstrated for numbers of kernel rows per cob irrespective of growing season. Difference between the genotypes was observed for cob length in spring, winter and combined genotype × season interaction (Table 1 and Table 2).

Grain yield has low heritability with combined mean of 6.07 t ha^{-1} (Table 2) but medium heritability on seasonal trial (Table 1). Here, winter maize produced 32% higher grain yield than spring maize. Differences were observed among the genotypes for grain yield on seasonal trials as well as combined over seasons. Top yielding PVA enriched bio-fortified maize hybrids such as HPO16-2, HPO49-3, HPO49-5, and HPO49-2 produced 38-61% higher grain yield than that of single cross normal hybrid check. Similarly, low-yielding genotypes such as HPO49-26 and HPO49-10 produced 15-20% lower grain yield than check hybrids.

Grain yield is a function of genotypes, environment (planting time, crop management etc.), and genotype × environment interaction. The analysis of variance for numbers of kernels rows cob-1, numbers of grains row-1 and grain yield revealed differences among the genotypes indicating the presence of genetic variability in the population studied. That result agrees with those reported earlier by Khan et al. (2018). Grain yield exhibited the highest positive genetic correlation with days to anthesis, days to silking, cob length, numbers of kernel rows, and numbers of grains per row, which is following the results of Vasic et al. (2001). Similar kinds of findings were also reported by Poudel, Paudel, and Yadav (2015). Therefore, selection for late flowering, taller plant height, longer cob length, more numbers of kernel rows and numbers of grains per row may produce higher grain yield in the populations studied. The higher grain yield of winter season planting as compared to spring season planting indicated that planting time is important for achieving higher grain yield. This might be due to longer duration of accumulation of solar radiation in winter maize than spring. Low temperature at the time of crop establishment and high temperature at reproductive stage are involved in comparatively lower grain yield of spring maize. These results are in line with Bakhtavar, Afzal, Basra, and Noor (2015) who reported effect of planting time on grain yield of maize. It showed that pro-vitamin A enriched maize also behaves like normal maize hybrids.

Table 3. Combined mean of Pro-vitamin A enriched maize hybrids and comparison of top yielding entries with others

EN	Genotypes	Days to 50%		Height (cm)		Cob length (cm)	No. of kernel rows	No. of grains per row	Grain yield (t ha^{-1})
		anthesis	silking	Plant	Ear				
29	HPO16-2	81	83	218	90	18.7	16.3	29.0	8.17
3	HPO49-3	76	77	219	107	18.3	14.9	36.7	7.88
5	HPO49-5	78	79	214	97	17.4	14.3	31.0	7.50
2	HPO49-2	81	83	202	97	18.5	14.4	32.8	7.04
30	RH-10 (Check)	81	83	239	124	17.3	14.2	28.3	5.09
26	HPO49-26	77	78	206	89	16.6	12.1	30.2	4.29
10	HPO49-10	78	80	195	75	16.9	13.6	28.4	4.05
Grand Mean		79	80	210	99	17.5	13.8	32.2	6.07
LSD0.05		2.29	2.86	14.3	13.09	1.38	1.00	2.64	1.18
Heritability		0.82	0.76	0.68	0.75	0.49	0.73	0.72	0.27
Genotype variance		**	**	**	**	ns	**	**	*
Gen × year variance		ns	*	ns	ns	**	ns	ns	ns

* significant at ≤0.05 p level,** significant at ≤0.01 p level, and ns=non-significant at 0.05 p level

Correlation between the traits

The correlation studies revealed reliable information on the nature, extent and direction of the selection. The knowledge of genetic correlation coefficient between quantitative characters and grain yield determines the efficiency of selection in maize breeding. The genotypic correlation highlights the true relationship, while phenotypic correlation reflects the observed relationships among the traits. Grain yield had positive and highly significant correlation with days to anthesis (r=0.99), days to silking (r=0.99), plant height (r=0.57), cob length (r=0.99), numbers of kernels per row (r=0.95), and numbers of grains per row (r=0.99) (Table 3). There was negative but significant association of plant height with days to anthesis (r=-0.29) and silking (r=-0.34). Plant height was positively correlated with ear height (r= 0.78) and numbers of kernel rows (r=0.46). A day to anthesis and silking was positively associated with cob length (r=0.48 & 0.52) and numbers of kernel rows (r=0.49 & 0.61) while numbers of grains per row was also positively correlated with cob length (r=0.39). The results also showed that cob length, numbers of grain per row and grain yield; days to anthesis, days to silking and numbers of kernel rows per cob; plant and ear height were related to each other. In agreement with the earlier findings of Zsubori et al. (2002), positive correlation observed between plant height and grain yield but negative correlation between plant height and days to flowering (anthesis and silking). The higher the ear height, the more ears can develop from the nodes below but if it is too high, ear may bend the stalk or even break it as suggested by Zsubori et al. (2002). Therefore, the position of the first cob must be below the middle of the plant for achieving good yield in maize hybrids.

Table 4. Genotypic correlations for maize grain yield and other agronomic characters combined over two seasons in Rampur

Traits	Days to anthesis	Days to silking	Plant height	Ear height	Cob length	No. of kernel rows	No. of grains per row
Days to silking	0.95**						
Plant height (cm)	-0.29*	-0.34*					
Ear height (cm)	0.08ns	0.28ns	0.78**				
Cob length (cm)	0.48*	0.52**	0.29ns	0.49*			
No. of kernel rows	0.49*	0.61**	0.46*	0.17ns	0.17ns		
No. of grains per row	-0.27ns	-0.33ns	-0.29ns	0.09ns	0.39*	-0.11ns	
Grain yield (kg ha-1)	0.99**	0.99**	0.57**	0.22ns	0.99**	0.95**	0.99**

* significant at ≤0.05 p level,** significant at ≤0.01 p level, and ns=non-significant at 0.05 p level

Conclusion

The study on pro-vitamin A enriched hybrid maize is an entirely new arena of research in the history of Nepal. Winter maize can produce a higher grain yield in the Terai and Inner Terai. Therefore, adjustment of planting time is important for achieving better yield from the varieties. HPO16-2, HPO49-3, HPO49-5, and HPO49-2 might be the potential future Pro-vitamin A enriched hybrids for Nepal. These hybrids might be the solution for solving the vitamin A deficiency prevailing among the children and women of Nepal. Further evaluation of these hybrids is necessary to generate data required for varietal release and bring greater awareness among the maize consuming communities about the nutritional benefits of PVA maize for scaling up. Finally, these hybrids might be part of the solutions for food insecurity, malnutrition, and a trade deficit of maize grains in the country.

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Author's declaration and contribution

The authors declare that there is no conflict of interest for the publication of this paper. MPT planed the research, gathered data, analyzed data and wrote this paper. DG gathered the data, wrote and edited this manuscript. KBK helped in data gathering, reviewing and advising. HKS and AB provided the genetic resources, supervised the research and reviewed results. All authors read the final manuscript and approved the final version.

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