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Double zero tillage and foliar phosphorus fertilization coupled with microbial inoculants enhance maize productivity and quality in a maize–wheat rotation

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Maize is an important industrial crop where yield and quality enhancement both assume greater importance. Clean production technologies like conservation agriculture and integrated nutrient management hold the key to enhance productivity and quality besides improving soil health and environment. Hence, maize productivity and quality were assessed under a maize–wheat cropping system (MWCS) using four crop-establishment and tillage management practices [FBCT–FBCT (Flat bed–conventional tillage both in maize and wheat); RBCT–RBZT (Raised bed–CT in maize and raised bed–zero tillage in wheat); FBZT–FBZT (FBZT both in maize and wheat); PRBZT–PRBZT (Permanent raised bed–ZT both in maize and wheat)], and five P-fertilization practices [P_{100} (100% soil applied-P); $P_{50} + 2FSP$ (50% soil applied-P + 2 foliar-sprays of P through 2% DAP both in maize and wheat); $P_{50} + PSB + AM$ -fungi; $P_{50} + PSB + AMF + 2FSP$; and P_0 (100% NK with no-P)] in split-plot design replicated-thrice. Double zero-tilled PRBZT–PRBZT system significantly enhanced the maize grain, starch, protein and oil yield by 13.1–19% over conventional FBCT–FBCT. $P_{50} + PSB + AMF + 2FSP$, integrating soil applied-P, microbial-inoculants and foliar-P, had significantly higher grain, starch, protein and oil yield by 12.5–17.2% over P_{100} besides saving 34.7% fertilizer-P both in maize and on cropping-system basis. $P_{50} + PSB + AMF + 2FSP$ again had significantly higher starch, lysine and tryptophan content by 4.6–10.4% over P_{100} due to sustained and synchronized P-bioavailability. Higher amylose content (24.1%) was observed in grains under $P_{50} + PSB + AMF + 2FSP$, a beneficial trait due to its lower glycemic-index highly required for diabetic patients, where current COVID-19 pandemic further necessitated the use of such dietary ingredients. Double zero-tilled PRBZT–PRBZT reported greater MUFA (oleic acid, 37.1%), MUFA: PUFA ratio and P/S index with 6.9% higher P/S index in corn-oil (an oil quality parameter highly required for heart-health) over RBCT–RBCT. MUFA, MUFA: PUFA ratio and P/S index were also higher under $P_{50} + PSB + AMF + 2FSP$; avowing the obvious role of foliar-P and microbial-inoculants in influencing maize fatty acid composition.

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Overall, double zero-tilled PRBZT–PRBZT with crop residue retention at 6 t/ha per year along with P₅₀ + PSB + AMF + 2FSP while saving 34.7% fertilizer-P in MWCS, may prove beneficial in enhancing maize productivity and quality so as to reinforce the food and nutritional security besides boosting food, corn-oil and starch industry in south-Asia and collateral arid agro-ecologies across the globe.

Abbreviations

AMF	Arbuscular mycorrhizal fungi
CA	Conservation agriculture
CETM	Crop establishment and tillage management
CPTs	Clean production technologies
CRR	Crop residue retention
C _{18:1}	Oleic acid
C _{18:2}	Linoleic acid
C _{16:0}	Palmitic acid
C _{18:0}	Stearic acid
CT	Conventional tillage
DAP	Di-ammonium phosphate
EC	Electrical conductivity
FBCT	Flat bed–conventional tillage
FBZT	Flat bed–zero tillage
2FSP	Two foliar sprays of phosphorus
ha	Hectare
IGPR	Indo-Gangetic Plains Region
K	Potassium
KHS	Knee-high stage in maize
m	Meter
m ha	Million hectares
Mt	Million tonnes
MWCS	Maize–wheat cropping system
MUFA	Monounsaturated fatty acid
N	Nitrogen
ODR	Oleic desaturation ratio
P	Phosphorus
P ₀	No-phosphorus
P ₅₀	50% recommended dose of P (as basal)
P ₁₀₀	100% recommended dose of P (as basal)
PUE	Phosphorus-use efficiency
PRBZT	Permanent raised bed–zero tillage
PSB	Phosphorus solubilizing bacteria
PUFA	Polyunsaturated fatty acid
PUE	Phosphorus-use efficiency
P/S index	PUFA/SFA ratio
RBCT	Raised bed–conventional tillage
RWCS	Rice–wheat cropping system
SFA	Saturated fatty acid
SOC	Soil organic carbon
t	Tonnes
TS	Tillering stage in wheat
UFA	Unsaturated fatty acid
ZT	Zero tillage

Under the aegis of United Nations Sustainable Development Goals (SDGs), there is an urgent need to focus both on food and nutritional quality enhancement for eradication of all types of hunger and malnutrition by 2030 especially in under-developed countries¹. We already know that rice–wheat cropping system (RWCS), a major system in south-Asia in general and India in particular, is a major contributor to the food and nutritional security of the region^{2,3}. However, intensive agriculture practices under RWCS especially in the Indo-Gangetic Plains Region (IGPR) coupled with intensive conventional tillage^{4,5}, sole use of chemical fertilizers^{6–8}, over-exploitation of groundwater⁹, and in situ crop residue burning^{10,11}, has led to stagnation in productivity with impaired quality, sub-soil compaction, soil health deterioration, groundwater depletion and gradual degradation of natural resource-base^{11,12}. The escalating labour, capital, and energy requirements⁴ coupled with receding groundwater table (~0.30–0.40 m year⁻¹)¹³, erratic rainfall pattern and intermittent droughts⁵, has further triggered the chronic fatigue in RWCS in south-Asian IGPR for over last three decades^{5,11}. Rice and wheat crops' residue burning has also long been a major cause of air pollution releasing huge gaseous emission in northern India^{10,14}, impairing soil and human health and environment¹⁵. To deter these ill-effects, crop diversification and conservation agriculture (CA) are two viable options^{4,16}, over the policy backed conventional RWCS¹⁷. Bringing *National Policy for Management of Crop Residues*¹⁸ in India is again a timely effort which stresses upon *in-situ* residue management through CA and other sustainable residue management methods¹⁹. Hence, research priorities integrating clean

production technologies (CPTs) viz. CA, best nutrient management practices and crop diversification should be set-up to avert these production- and resource vulnerabilities in IGPR^{17,20,21}. Overall, this study heeds to the SDGs of the United Nations (with respect to land degradation neutrality and land restoration) from exploitation to the sustainable use of resources^{22,23}, and soil health management^{24,25}; so as to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture as per SDG2²⁶. The climate-resilient conservation agriculture which follows minimal soil disturbance, crop residue retention and crop rotations^{6,27}, have been advocated to significantly improve the soil organic matter and soil health in holistic manner besides enhanced resource-use efficiency and crop yields in the vulnerable agro-ecologies across the globe^{8,21,28–35}. Since, maize is one of the important cereal crops in south-Asia after rice and wheat, with the consumption of ~ 39.4 Mt maize grains in the region where India alone consumes ~ 24 Mt maize grains³⁶. Hence, in order to safeguard the food security of millions of south-Asian families, maize farming tailored with CA practices followed in maize–wheat cropping system (MWCS) may prove as viable alternative to diversify the RWCS and boosting the productivity while concurrently conserving the soil, environment and natural resources^{21,27,37}.

Globally, maize (*Zea mays* L.) is grown in ~ 193.7 m ha area producing ~ 1147.6 Mt grains with an average yield of 5.92 t ha⁻¹³⁶. Alone in India, maize is grown on ~ 9.2 m ha area producing 27.8 Mt grains but with poor productivity ~ 3.05 t ha⁻¹³⁶, and quality³⁸. Maize is popularly known as queen of the cereals because of its high yield potential and wider adaptability to diverse agro-ecologies³⁹. Maize is a vital crop for food and nutritional security in world's poorest regions in Asia, Africa and Latin America^{1,40}. Worldwide, maize is consumed in ~ 94 developing countries comprising > 4.5 billion people where it supplies ~ 30% of total calorie needs. Maize grain is a good source of high quality starch⁴¹; while its oil contains essential polyunsaturated fatty acids (PUFAs) that are highly beneficial in the management of cardiovascular diseases due to their vital role in blood cholesterol regulation and lowering of elevated blood pressure^{38,42}. Maize is a versatile industrial crop processed into various food and non-food products viz. starch, vegetable oil, sweeteners, beverages, glue, alcohol and bioethanol, etc. Alone in India, ~ 20% of total maize consumption is utilized for non-food industrial product development, ~ 14% of which is solely utilized in starch manufacturing for pharmaceutical, textile, paper and food industry uses⁴³. Likewise, higher amylose content in maize grains is a beneficial trait as it contains resistant starch (RS) type-2 with low glycemic index⁴⁴, which is high demand in food industry for diabetic patients⁴⁵. Current COVID-19 pandemic has further necessitated the management of this major comorbidity factor (diabetes) using such dietary ingredients⁴⁶. For meeting global edible oil demands, maize is again a vital alternative containing ~ 3–4% oils in maize germ, an oil-rich part of maize kernel^{47,48}. Corn oil is a rich source of linoleic acid (essential fatty acid), which is one of the two essential acids necessary for the integrity of the skin, cell membranes and immune system and for synthesis of eicosanoids necessary for reproductive, cardiovascular, renal, gastrointestinal functions and resistance to the diseases besides being highly effective in lowering the serum cholesterol primarily low-density-lipoprotein cholesterol⁴⁹. Overall, maize grains with higher starch, oil and protein content are in high demand in food and non-food industry^{43,50}. However, lysine and tryptophan are the deficient amino acids in cereals like maize which are essential for making the building blocks of human body. The crop productivity and quality can be increased through appropriate best plant nutrition¹⁷, bio-fortification approaches⁵¹, and agronomic practices like CA^{33,52,53} besides using breeding tools⁵⁴. Hence, maize productivity and quality enhancement through agronomic approaches assumes utmost importance in curtailing the hunger and malnutrition besides promoting its industrial usages⁵⁵. As, ~ 73% of total global maize area is located in developing world, hence, improved productivity and quality traits through low-cost CPTs may open new vistas for maize growers and agri-entrepreneurs to fetch higher prices for quality maize produce in food and industrial sectors.

Phosphorus (P) is one of the most important nutrient elements which plays an important role in enhancing the productivity and quality while influencing various plant processes like energy storage and transfer, photosynthesis, root growth, flowering, seed setting and seed yield, etc.^{56–58}. Due to poor native-P status, low solubility and low efficiency ~ 10–20% across the majority of global arable soils, the P is a critical nutrient that greatly limits plant growth, yield and quality^{57,59}. In order to improve the productivity, quality and P-use efficiency (PUE) in this high nutrient requiring crop due to its high yield potential, devising efficient P management strategies with integration of soil applied P-fertilizers, biofertilizers and innovative approaches like foliar-P fertilization that too under CA based systems, may assume utmost importance. Alluvial soils in Indian IGPR are characterized as most fertile soils but now majority of them are diagnosed with low soil-P status⁶⁰. The P-fertilizers are already very costly and the most of the soil applied-P gives low PUE with fate of native and applied-P being fixed as Ca and Mg phosphate in alkaline soils of IGPR⁶⁰. Phosphorus-solubilizing bacteria (PSB) and AM-fungi also hold great potential in solubilization and mobilization of native and applied-P^{51,61}. Foliar P-fertilization has also shown positive influence on crop productivity and quality in many crops^{58,62}. Thus, foliar-P fertilization along with microbial inoculants may prove as a low-cost CPT in nutrient exhaustive crops like maize to harness higher yield with better quality and PUE besides saving soil applied-P. However, the impacts of conservation agriculture coupled with this innovative P-management strategy integrating soil applied P, foliar-P and microbial inoculants in maize–wheat cropping system are yet to be evaluated with respect to maize productivity and quality parameters (starch, protein, amino acid and fatty acid composition) that too under South-Asian semi-arid climate. Overall, the CPTs like conservation agriculture along with foliar-P fertilization may enhance both productivity and quality to augment its safe food and industrial uses besides improving soil health and environment. However, no systematic research work has been carried-out till date to assess the impact of such climate-resilient CPTs especially CA and the innovative foliar-P fertilization on quality parameters of maize in a semi-arid agro-ecology. Therefore, this study assessed the impacts of the CA based crop establishment and tillage management (CETM) and microbial inoculants' imbedded P-fertilization practices on maize yield and grain quality under MWCS so as to scale-up the food and nutrition security under the precept of United Nations SDGs, besides augmenting its safe food and industrial uses in blooming food, starch and corn-oil industry in south-Asia.

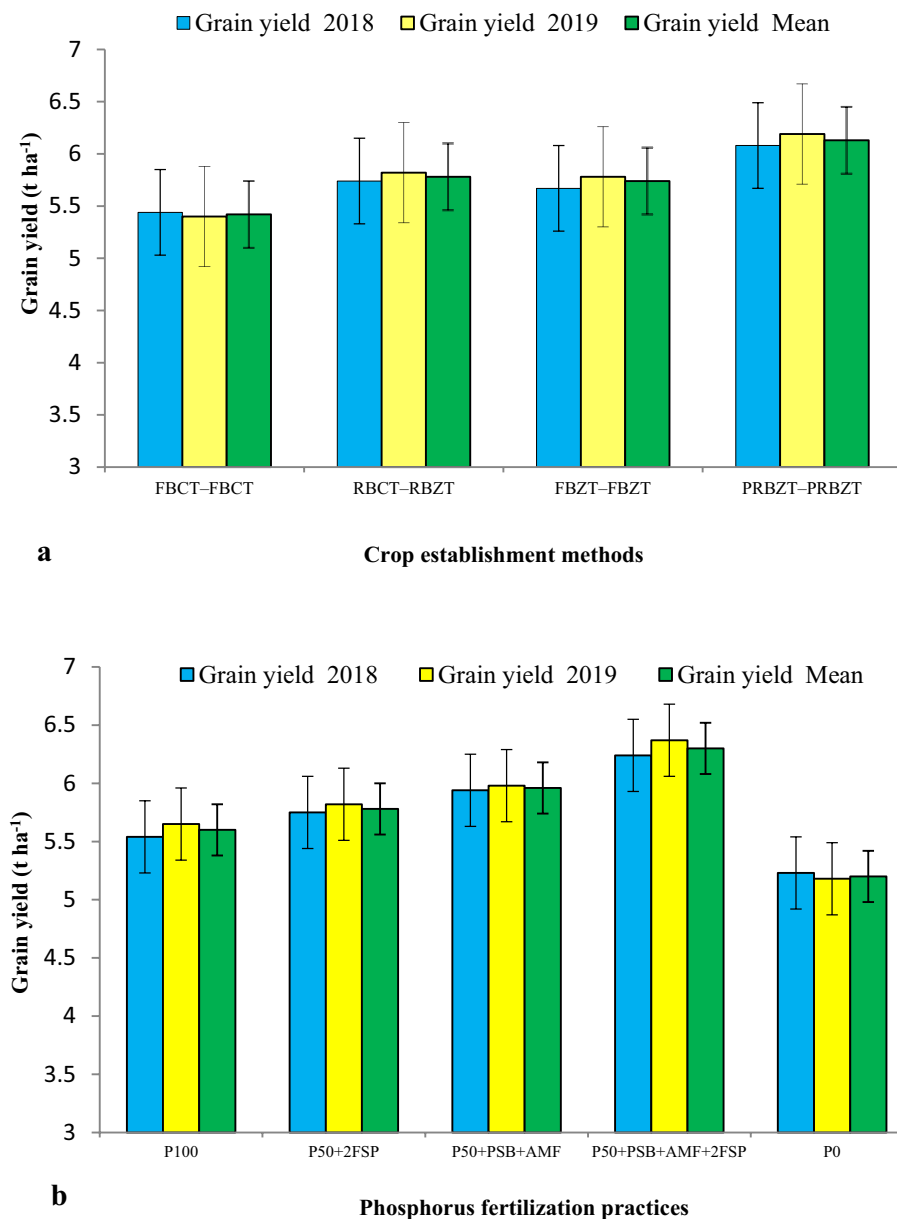


Figure 1. Influence of (a) CETM and (b) P-fertilization practices on grain yield (t ha^{-1}) of maize under MWCS. The vertical bars represent $\text{LSD}_{0.05}$ values.

Results

Maize grain yield. Maize grain yield was significantly ($p < 0.05$) influenced by the crop establishment and tillage management practices (CETMs) as well as P-fertilization practices (PFPs) during both years (Fig. 1). The double zero-tilled permanent raised-beds with crop residue retention of 6 t ha^{-1} per year under treatment PRBZT-PRBZT in MWCS resulted in significantly higher mean maize grain yield (6.13 t ha^{-1}) by 6.4, 5.7 and 13.1% over RBCT-RBZT, FBZT-FBZT and FBCT-FBCT. The integration of 50% P + PSB + AMF + 2FSP, a combination of soil applied-P, microbial inoculants and the two foliar-P sprays (2% DAP), observed significantly ($p < 0.05$) higher grain yield (6.3 t ha^{-1}) by 5.4, 8.3, 11.3 and 17.5% over P_{50} + PSB + AMF, P_{50} + 2FSP, P_{100} and P_0 , respectively. However, P_{50} + 2FSP, P_{50} + PSB + AMF and P_{100} treatments were statistically at par with each other. The interaction effects between the CETMs and PFPs were found significant in the current study (Supplementary Table S1). On an average, PRBZT-PRBZT and P_{50} + PSB + AMF + 2FSP exhibited ~13.1 and 11.3% higher maize grain yield over their respective counterpart treatments FBCT-FBCT (conventional-tilled FB system both in maize and wheat) and the P_{100} (100% soil applied-P), respectively (Fig. 1).

Starch content and starch yield. The CETMs exhibited higher magnitude of starch content (65.1–67.2%) in maize grains under CA plots (PRBZT-PRBZT, FBZT-FBZT, RBCT-RBZT) compared to 64.4% in the conventional-tilled FB system (FBCT-FBCT) (Table 1). Likewise, maize under raised-beds (PRBZT-PRBZT,

Treatment	Starch (%)			Starch yield (kg ha ⁻¹)			Protein content (%)			Protein yield (kg ha ⁻¹)		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
CETM practices												
FBCT–FBCT	64.5	64.2	64.4	3511	3494	3503	8.85	8.91	8.88	481.4	483.0	482.2
RBCT–RBZT	65.5	64.6	65.1	3774	3836	3805	9.18	9.22	9.20	527.8	538.2	533.0
FBZT–FBZT	65.5	65.3	65.4	3752	3798	3775	9.12	9.38	9.25	520.6	543.3	531.9
PRBZT–PRBZT	67.4	67.0	67.2	4113	4202	4157	9.17	9.50	9.33	558.3	589.5	573.9
CD ($p=0.05$)	NS	NS	NS	362	438	307	NS	0.30	0.3	34.0	49.4	29.1
P-fertilization practices												
P ₁₀₀	66.3	65.9	66.1	3681	3767	3724	9.10	9.35	9.23	505.2	528.0	516.6
P ₅₀ + 2FSP	67.0	67.1	67.0	3854	3914	3884	9.30	9.62	9.46	535.9	560.5	548.2
P ₅₀ + PSB + AMF	64.9	65.3	65.1	3855	3906	3881	9.23	9.43	9.33	548.2	563.6	555.9
P ₅₀ + PSB + AMF + 2FSP	68.6	69.2	68.9	4288	4413	4350	9.48	9.70	9.59	591.9	619.8	605.6
P ₀	62.1	61.0	61.5	3260	3163	3211	8.28	8.15	8.22	428.9	421.2	425.0
CD ($p=0.05$)	4.3	4.2	4.1	354	358	321	0.49	0.43	0.45	30.9	32.8	24.4
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS

Table 1. Influence of CETM and P-fertilization practices on grain starch content (%), starch yield (kg ha⁻¹), protein content (%) and protein yield (kg ha⁻¹) in maize under MWCS. CD values indicate the critical difference at $p=0.05$.

RBCT–RBZT) exhibited higher starch content (~66.2%) compared to ~64.9% under flat-beds (FBZT–FBZT, FBCT–FBCT) irrespective of the tillage practices.

Among PFPs, significantly ($p < 0.05$) higher starch content (68.9%) were obtained by applying P₅₀ + PSB + AMF + 2FSP followed by P₅₀ + 2FSP (67.1%) and least under P₀ (61.6%). Despite of non-significant effect of CETMs on starch content, the starch yield was significantly ($p < 0.05$) influenced by both CETMs and PFPs with significantly ($p < 0.05$) higher values under PRBZT–PRBZT (4157 kg ha⁻¹), a double zero-tilled PRB system, and the P₅₀ + PSB + AMF + 2FSP (4350 kg ha⁻¹), a combination of soil applied-P, microbial inoculants and the two foliar-P sprays (Table 1). On an average, PRBZT–PRBZT and P₅₀ + PSB + AMF + 2FSP reported ~18.6 and 16.8% higher starch yield over their respective counterpart treatments FBCT–FBCT and P₁₀₀, respectively (Table 1).

Amylose and amylopectin content. The CETMs did not show any significant influence on the amylose content while PFPs exhibited significant effect ($p < 0.05$) on the amylose content in maize grains during both years (Fig. 2). Under CETMs, highest amylose content (23%) were reported under PRBZT–PRBZT while other treatments exhibited ~21.2–21.5% amylose content. Integration of P₅₀ + PSB + AMF + 2FSP resulted in significantly ($p < 0.05$) higher amylose content (24.1%) which was followed by P₁₀₀, P₅₀ + 2FSP, P₅₀ + PSB + AMF and P₀, respectively (Fig. 2). On an average, PRBZT–PRBZT and P₅₀ + PSB + AMF + 2FSP exhibited ~6.5 and 1.7% higher amylose content over the FBCT–FBCT and P₁₀₀, respectively. The PFPs again exhibited significant effect ($p < 0.05$) on amylopectin content while CETMs did not show any significant influence on amylopectin content during both years (Fig. 2). The amylopectin followed the reverse trend as that of amylose content both for CETMs and PFPs. Highest amylopectin content was achieved under RBCT–RBZT (78.9%) and least under PRBZT–PRBZT (77.1%). The P₀ exhibited significantly ($p < 0.05$) higher amylopectin content (81.5%) while P₅₀ + PSB + AMF + 2FSP exhibited least values (75.9%).

Protein content and protein yield. The CETMs had non-significant influence on protein content in maize grains during first year; however the effects were significant ($p < 0.05$) during second year (Table 1), where CA-based CETMs (PRBZT–PRBZT, FBZT–FBZT, RBCT–RBZT) had higher protein content (9.2–9.33%) compared to FBCT–FBCT (8.88%). Maize grown on raised-beds (PRBZT–PRBZT, RBCT–RBZT) exhibited higher protein content compared to flat-beds (FBZT–FBZT, FBCT–FBCT) during both years. Double zero-tilled PRBZT–PRBZT system produced ~5.1% higher protein content compared to conventional-tilled FBCT–FBCT system. Among PFPs, P₅₀ + PSB + AMF + 2FSP exhibited significantly ($p < 0.05$) higher protein content (9.59%) which was followed by P₅₀ + 2FSP with lowest magnitude under P₀ (9.46%) during the study. The integration of P₅₀ + PSB + AMF + 2FSP enhanced the protein content by ~3.9% over the recommended PFP (P₁₀₀). The CETMs and PFPs both had significant ($p < 0.05$) influence on protein yield following the similar trend as that of grain yield where PRBZT–PRBZT and P₅₀ + PSB + AMF + 2FSP both produced significantly superior protein yield during both years (Table 1). However, the interaction effects between the CETMs and PFPs for the protein yield were found to be significant only during second year (Supplementary Table S2). On an average, PRBZT–PRBZT and P₅₀ + PSB + AMF + 2FSP exhibited ~19 and 17.2% higher protein yield over their respective counterpart treatments viz. FBCT–FBCT and P₁₀₀, respectively (Table 1).

Lysine and tryptophan content. The CETMs did not have any significant effect on the lysine content in maize grains, however, the raised-bed CETMs viz. PRBZT–PRBZT and RBCT–RBZT produced higher lysine

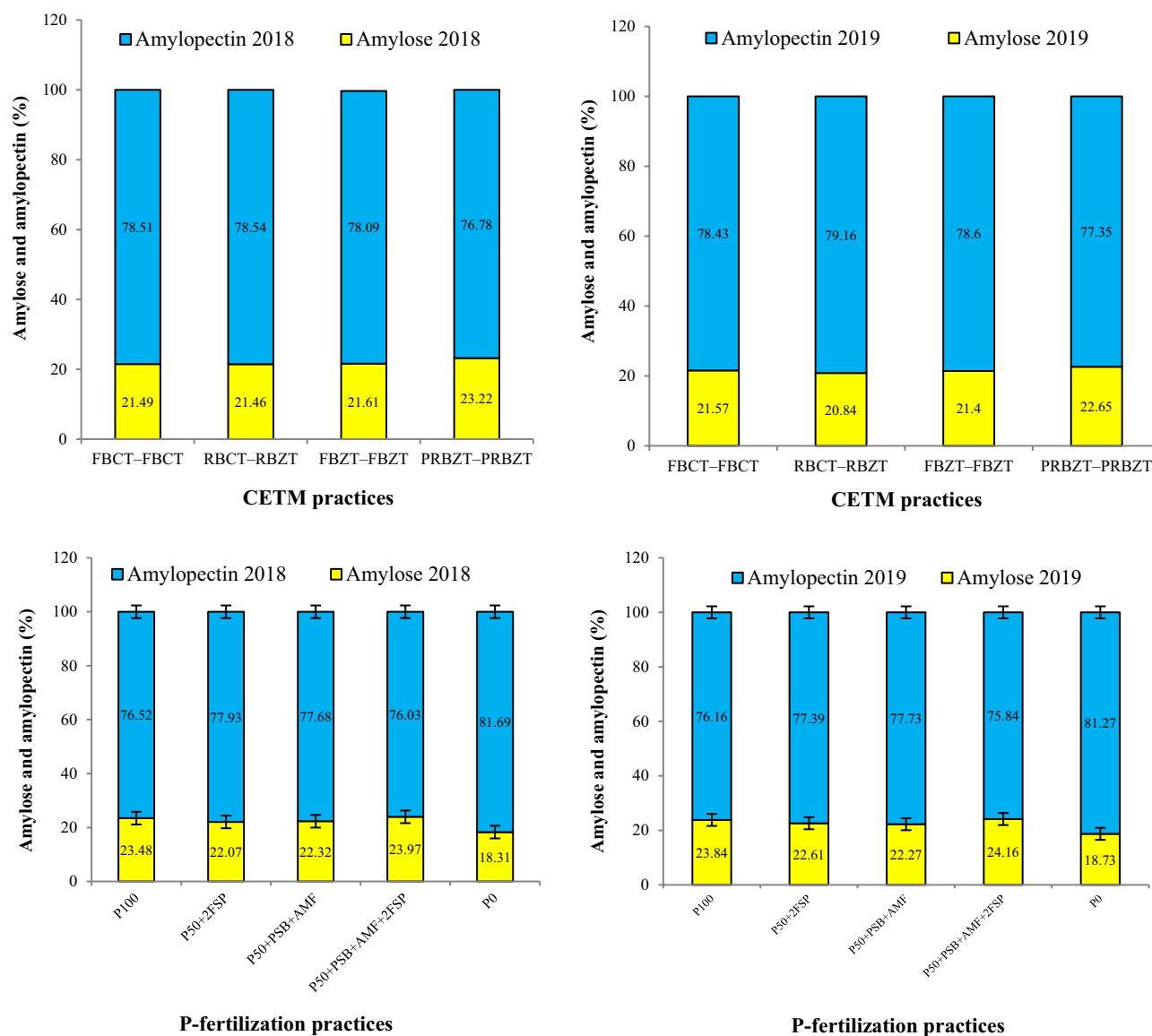


Figure 2. Influence of CETM and P-fertilization practices on grain amylose and amylopectin content of maize under MWCS. The vertical bars represent $LSD_{0.05}$ values.

content over their flat-bed counterpart CETMs viz. FBZT-FBZT and FBCT-FBCT, respectively (Table 2). The PRBZT-PRBZT observed ~1.2% higher lysine content over the FBCT-FBCT. The $P_{50} + PSB + AMF + 2FSP$ reported significantly higher lysine content (2.74 g kg^{-1} dry matter) which was followed by P_{100} , $P_{50} + 2FSP$, $P_{50} + PSB + AMF$ and P_0 , respectively (Table 2). Integration of $P_{50} + PSB + AMF + 2FSP$ exhibited ~6.6% higher lysine content over 100% soil applied-P. The CETMs again didn't show any significant influence on tryptophan content, although, double zero-tilled PRBZT-PRBZT and FBZT-FBZT treatments reported comparatively higher tryptophan content over the conventional-tilled FBCT-FBCT system. Among PFPs, $P_{50} + PSB + AMF + 2FSP$ had significantly higher tryptophan content by ~10.4% over P_{100} . In general, tryptophan followed similar trend as that of protein content both for CETMs and PFPs in current study (Table 2).

Oil content and oil yield. Effect of CETMs on corn-oil content was found non-significant (Table 3). However, double zero-tilled PRBZT-PRBZT (4.83%) and FBZT-FBZT (4.74%) reported higher corn-oil content over the single-crop based zero-tilled RBCT-RBZT (4.65%) and no-tilled FBCT-FBCT (4.63%). Likewise, maize under raised-beds (PRBZT-PRBZT, RBCT-RBZT) exhibited higher oil content over their counterpart flat-bed CETMs (FBZT-FBZT, FBCT-FBCT) irrespective of the tillage followed. On an average, double zero-tilled PRBZT-PRBZT system realized ~4.3% higher oil content over the CT based FBCT-FBCT. Among PFPs, significantly ($p < 0.05$) higher oil content was obtained by applying $P_{50} + PSB + AMF + 2FSP$ (4.9%) followed by P_{100} (4.84%) and least under P_0 (4.41%). The $P_{50} + PSB + AMF + 2FSP$ enhanced the oil content by ~1.2 and 11.1% over P_{100} and P_0 , respectively. Oil yield was significantly influenced by both CETM and PFPs with significantly ($p < 0.05$) higher magnitude under PRBZT-PRBZT (297.2 kg ha^{-1}) and $P_{50} + PSB + AMF + 2FSP$ (309.4 kg ha^{-1}) to the tune of ~18.1 and 14.3% over their respective counterpart treatments viz. FBCT-FBCT and P_{100} (Table 3).

Treatment	Lysine content (g per kg dry matter)			Tryptophan ($\mu\text{g g}^{-1}$)			P uptake by grains (kg ha^{-1})		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
CETM practices									
FBCT–FBCT	2.45	2.44	2.44	0.64	0.65	0.64	14.92	15.45	15.18
RBCT–RBZT	2.48	2.46	2.47	0.64	0.66	0.65	16.30	16.77	16.53
FBZT–FBZT	2.44	2.48	2.46	0.66	0.66	0.66	14.54	17.35	15.94
PRBZT–PRBZT	2.48	2.47	2.47	0.65	0.67	0.66	18.28	19.03	18.65
SEm \pm	0.02	0.03	0.03	0.02	0.02	0.02	0.60	0.38	0.41
CD ($p=0.05$)	NS	NS	NS	NS	NS	NS	2.01	1.31	1.41
P-fertilization practices									
P ₁₀₀	2.55	2.59	2.57	0.65	0.67	0.67	14.78	16.52	15.65
P ₅₀ + 2FSP	2.47	2.50	2.48	0.72	0.73	0.72	16.46	17.81	17.13
P ₅₀ + PSB + AMF	2.45	2.42	2.44	0.66	0.69	0.68	17.18	17.29	17.24
P ₅₀ + PSB + AMF + 2FSP	2.72	2.75	2.74	0.74	0.75	0.74	18.73	20.98	19.86
P ₀	2.11	2.06	2.09	0.45	0.43	0.44	12.88	13.14	13.01
SEm \pm	0.06	0.06	0.06	0.02	0.02	0.02	0.72	0.57	0.51
CD ($p=0.05$)	0.18	0.17	0.18	0.04	0.05	0.04	2.06	1.64	1.47
Interaction	NS	NS	NS	NS	NS	NS	S	S	S

Table 2. Influence of CETM and P-fertilization practices on lysine (g per kg dry matter), tryptophan content ($\mu\text{g g}^{-1}$) and grain-P uptake (kg ha^{-1}) of maize under MWCS. CD values indicate the critical difference at $p=0.05$.

Treatment	Oil content (%)			Oil yield (kg ha^{-1})		
	2018–19	2019–20	Mean	2018–19	2019–20	Mean
CETM practices						
FBCT–FBCT	4.62	4.64	4.63	251.3	252.0	251.7
RBCT–RBZT	4.63	4.66	4.65	266.6	272.3	269.4
FBZT–FBZT	4.73	4.75	4.74	269.3	274.2	271.8
PRBZT–PRBZT	4.81	4.86	4.83	292.7	301.7	297.2
CD ($p=0.05$)	NS	NS	NS	25.09	18.56	15.00
P-fertilization practices						
P ₁₀₀	4.81	4.86	4.84	266.8	274.6	270.7
P ₅₀ + 2FSP	4.79	4.82	4.81	276.2	281.3	278.7
P ₅₀ + PSB + AMF	4.59	4.61	4.60	272.9	275.6	274.2
P ₅₀ + PSB + AMF + 2FSP	4.86	4.95	4.90	303.0	315.7	309.3
P ₀	4.43	4.40	4.41	231.2	228.0	229.6
CD ($p=0.05$)	0.25	0.24	0.25	21.55	22.56	19.7
Interaction	NS	NS	NS	NS	NS	NS

Table 3. Influence of CETM and P-fertilization practices on oil content (%) and oil yield (kg ha^{-1}) of maize under MWCS. CD values indicate the critical difference at $p=0.05$.

Fatty acid profiling. Effect of CETMs on fatty acid content in maize grain oil was found non-significant (Fig. 3). However, the saturated fatty acid (SFA) (Palmitic acid + Stearic acid) and poly unsaturated fatty acid (PUFA) (Linoleic acid) content, were higher under conventionally-tilled plots (RBCT–RBZT, FBCT–FBCT) compared to double zero-tilled plots (PRBZT–PRBZT, FBZT–FBZT). A reverse trend was observed for mono unsaturated fatty acid (MUFA) content (Oleic acid) where double zero-tilled CETMs (PRBZT–PRBZT, FBZT–FBZT) exhibited higher MUFA (Oleic acid) content over the conventionally-tilled RBCT–RBZT and FBCT–FBCT CETMs. The PRBZT–PRBZT exhibited highest MUFA (oleic acid) content (37.3%) while FBZT–FBZT had highest PUFA (Linoleic acid) content (48.4%). The PFPs showed significant effect on PUFA and MUFA composition except SFA (Palmitic acid + Stearic acid). The SFA and PUFA content were higher under P₀ (16.7; 48.4%) and lowest under P₅₀ + PSB + AMF + 2FSP (15.5; 45.7%), respectively; whereas MUFA content were higher under P₅₀ + PSB + AMF + 2FSP (38.5%) and least under P₀ (34.1%); exhibiting the obvious role of PFPs in influencing fatty acid concentration in corn-oil. No-P supply (P₀) resulted in inhibitory effect on MUFA (Oleic acid) content and resulted in higher SFA (Palmitic and Stearic acid) and PUFA (Linoleic acid) content. Thus, MUFA and PUFA content differed significantly due to PFPs. The SFA and PUFA followed the trend of P₀ > P₅₀ + PSB + AMF > P₅₀ + 2FSP > P₁₀₀ with respective higher values (16.7; 48.4%) under P₀ and lowest values

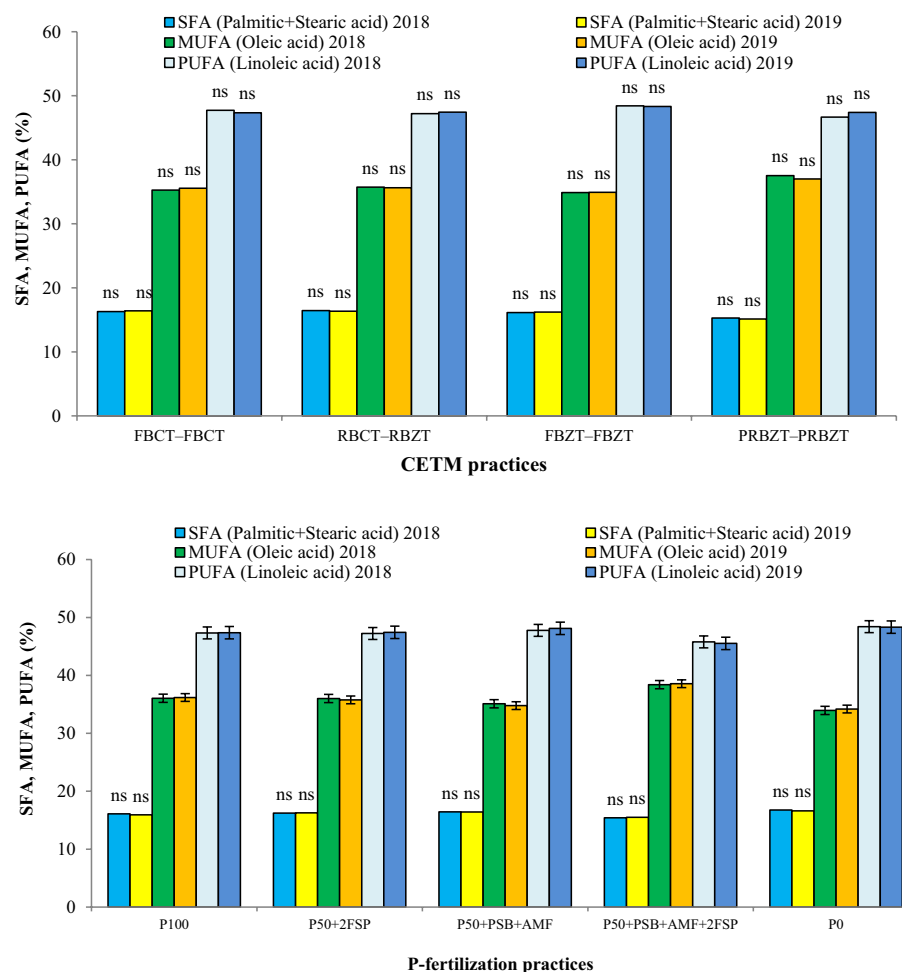


Figure 3. Influence of CETM and P-fertilization practices on fatty acid composition viz. SFA [Saturated fatty acid (Palmitic acid + Stearic acid)], MUFA [Mono unsaturated fatty acid (Oleic acid)] and PUFA [Poly unsaturated fatty acid (Linoleic acid)] in maize under MWCS. The vertical bars represent $LSD_{0.05}$ values.

(15.5; 45.7%) under $P_{50} + PSB + AMF + 2FSP$, respectively. The MUFA content showed reverse trend with higher values under $P_{50} + PSB + AMF + 2FSP$ (38.5%) and least under P_0 (34.1%). On an average, PRBZT-PRBZT and $P_{50} + PSB + AMF + 2FSP$ exhibited ~5.2 and 6.5% higher MUFA (Oleic acid) content over their respective counterpart treatments viz. FBCT-FBCT and P_{100} (Fig. 3).

Fatty acid ratios. Fatty acid ratios in corn-oil viz. oleic desaturation ratio (ODR), MUFA: PUFA ratio, SFA: unsaturated fatty acid ratio (SFA: UFA ratio) and P/S index showed considerable variations for CETMs and PFPs (Fig. 4). The ODR and SFA: UFA ratio didn't show any significant differences while MUFA: PUFA ratio and P/S index exhibited significant differences under CETMs. Double zero-tilled PRBZT-PRBZT had highest MUFA: PUFA ratio (0.79) and P/S index (3.09) while other CETMs were statistically similar amongst them. The ODR, MUFA: PUFA ratio and P/S index responded positively and significantly ($p < 0.05$) to PFPs (Fig. 4). The P_0 had highest ODR (0.59) but with least MUFA: PUFA ratio (0.70) and P/S index (2.9). The $P_{50} + PSB + AMF + 2FSP$ had least ODR (0.54) but with highest MUFA: PUFA ratio while remaining PFPs were statistically similar amongst them for ODR and MUFA: PUFA ratio. Highest P/S index was found under P_{100} (2.96) followed by $P_{50} + PSB + AMF + 2FSP$, $P_{50} + PSB + AMF$ and $P_{50} + 2FSP$, respectively. On an average, double zero-tilled PRBZT-PRBZT and soil-applied P_{100} exhibited ~6.9 and 2.1% higher P/S index in corn-oil over their respective counterpart treatments viz. RBCT-RBCT and P_0 (Fig. 4).

Maize grain P-uptake. The maize grain P-uptake followed the trend of PRBZT-PRBZT > RBCT-RBCT > FBZT-FBZT > FBCT-FBCT with significantly higher values under double zero-tilled PRBZT-PRBZT system (18.7 $kg\ ha^{-1}$) (Table 2). Among PFPs, integrated use of $P_{50} + PSB + AMF + 2FSP$ led to significantly ($p < 0.05$) higher grain P-uptake, though it was statistically similar to P_{100} following the trend $P_{50} + PSB + AMF + 2FSP > P_{50} + PSB + AMF > P_{50} + 2FSP > P_{100} > P_0$ (Table 2). On an average, double zero-tilled

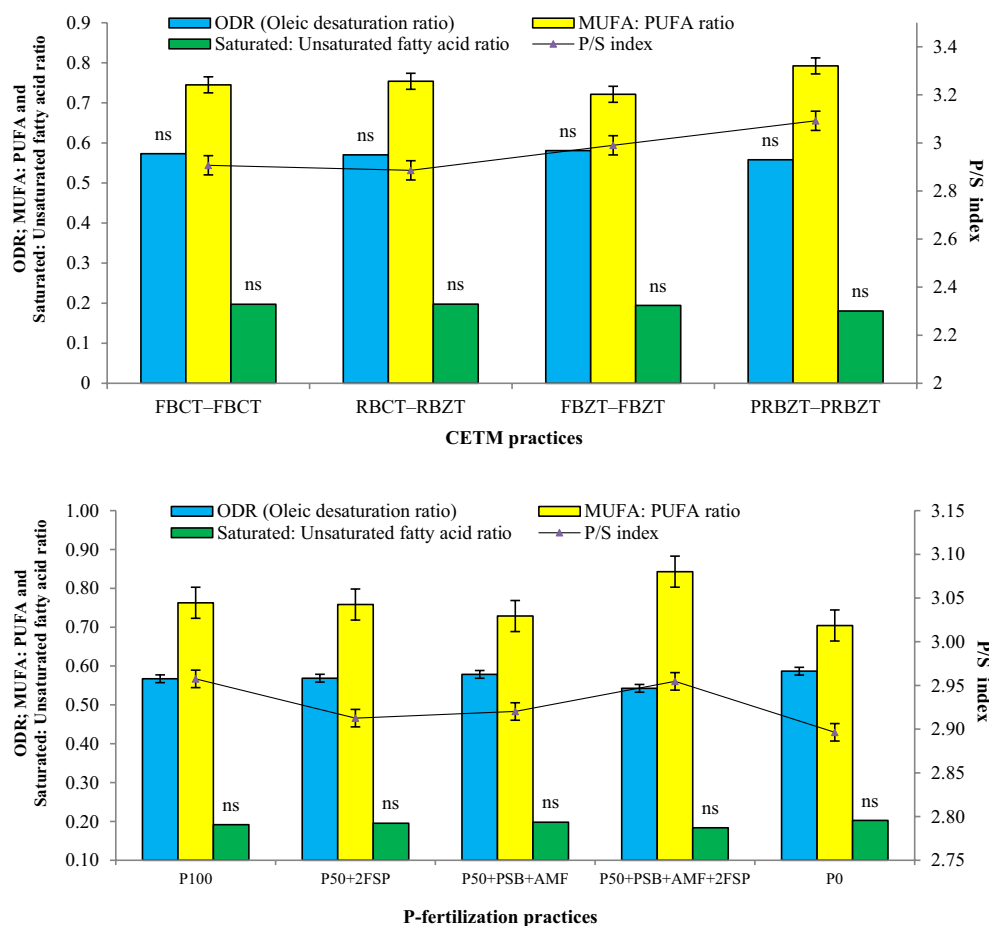


Figure 4. Influence of CETM and P-fertilization practices on various fatty acid ratios of maize under MWCS. The vertical bars represent $LSD_{0.05}$ values.

PRBZT-PRBZT and $P_{50} + PSB + AMF + 2FSP$ exhibited ~22.9 and 26.9% higher grain P-uptake over their respective counterpart treatments viz. FBCT-FBCT and P_{100} .

Correlation studies. Starch content in maize grains showed positive correlation ($p < 0.05$) with grain yield both for CETMs ($R^2 = 0.921$) and PFPs ($R^2 = 0.756$) (Figs. 5, 6). Starch content again showed positive correlation ($p < 0.05$) with grain P-uptake ($R^2 = 0.852$) under PFPs (Fig. 7). Amylose content had positive correlation ($R^2 = 0.54$; 0.606), while amylopectin had negative association ($R^2 = -0.54$; -0.635) with grain yield both for CETMs and PFPs, respectively (Figs. 5, 6). Amylose content again had positive association ($R^2 = 0.658$) while amylopectin had negative correlation ($R^2 = -0.676$) with grain P-uptake under PFPs (Fig. 7). More to the point, protein content showed positive correlation with grain yield both under CETMs ($R^2 = 0.832$) and PFPs ($R^2 = 0.774$) as well as with grain P-uptake ($R^2 = 0.828$) under PFPs (Figs. 5, 6, 7). Lysine ($R^2 = 0.754$; 0.742) and tryptophan content ($R^2 = 0.669$; 0.741) had positive correlation with grain yield both for CETMs and PFPs, respectively (Figs. 5, 6).

Likewise, lysine ($R^2 = 0.795$) and tryptophan content ($R^2 = 0.802$) showed positive correlation with grain P-uptake under PFPs (Fig. 7). Oil content had strong positive association ($R^2 = 0.76$) with grain yield under CETMs (Fig. 5), but a moderate positive correlation ($R^2 = 0.476$) under PFPs (Fig. 6). Oil content showed a moderate positive correlation with grain P-uptake ($R^2 = 0.584$) under PFPs (Fig. 7). Among various fatty acids, only MUFA had positive correlation ($p < 0.05$) with grain P-uptake ($R^2 = 0.726$) while SFA ($R^2 = -0.676$) and PUFA ($R^2 = -0.724$) had negative relationship with grain P-uptake under PFPs (Fig. 7). Grain P-uptake and MUFA: PUFA ratio had a positive correlation ($R^2 = 0.723$), whereas ODR ($R^2 = -0.729$) and SFA: UFA ratio ($R^2 = -0.684$) had negative correlation with grain P-uptake under PFPs (Fig. 7).

Principal component analysis and clustered heatmap. Principal component analysis (PCA) revealed the differences in the composition of maize quality parameters under different combinations of CETMs and PFPs ($p < 0.05$). As shown in Fig. 8, all the treatment combinations clustered distinctly. Treatment combinations M_4S_3 (PRBZT-PRBZT coupled with $P_{50} + PSB + AMF$) and M_3S_4 (FBZT-FBZT coupled with $P_{50} + PSB + AMF + 2FSP$) exhibited higher amylose, protein, lysine, tryptophan and oil content. Likewise, M_4 (PRBZT-PRBZT), M_3 (FBZT-FBZT) and M_2 (RBCT-RBZT) in combination with S_4 ($P_{50} + PSB + AMF + 2FSP$), S_3 ($P_{50} + PSB + AMF$) and S_2

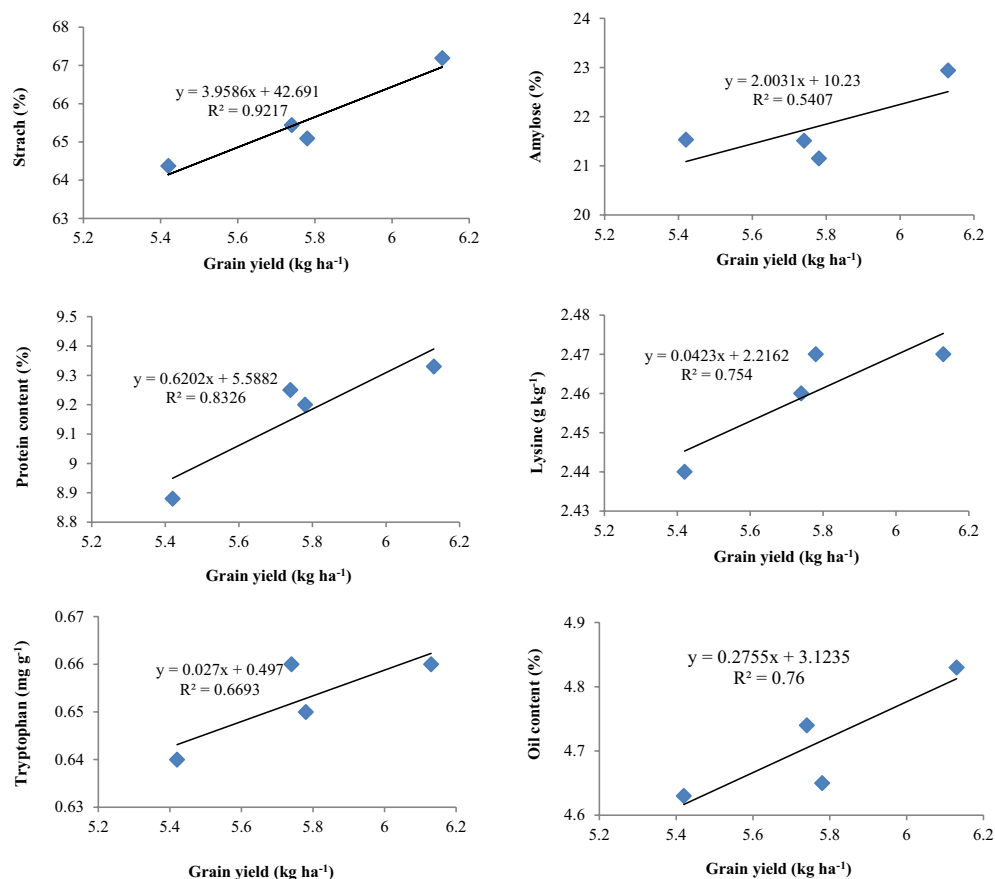


Figure 5. Correlation between grain yield and quality parameters of maize under CETM practices in maize under MWCS.

(P₅₀ + 2FSP) had a positive correlation with both component one and component two, exhibiting increased grain yield, protein yield, oil yield and starch content. The conventionally-tilled M₁ (FBCT–FBCT) along with S₅ (P₀) exhibited higher amylopectin content than other treatment combinations (Fig. 8). For a better understanding of the clustering pattern of grain yield and quality parameters across the treatment combinations; a biclustering heatmap was generated (Fig. 9). This heatmap showed that maize yield and quality parameters (except amylopectin) clustered closely and displayed an increase under CA based CETMs (M₄, M₃ and M₂) in combination with the PFPs viz. S₄ (P₅₀ + PSB + AMF + 2FSP), S₃ (P₅₀ + PSB + AMF) and S₂ (P₅₀ + 2FSP). Most significant and remarkable shifts were found for amylopectin content where conventionally-tilled M₁ clustered closer to the CA based CETMs (M₄, M₃ and M₂) all supplied with no-P. On an average, CA based CETMs (M₄ and M₃) in combination with S₄ (P₅₀ + PSB + AMF + 2FSP) and S₂ (P₅₀ + 2FSP) showed higher similarity to each other and formed a cluster for maize yield and the majority of the quality parameters. The M₄S₄ (CA based PRBZT–PRBZT supplied with P₅₀ + PSB + AMF + 2FSP) was proved as best treatment combination for realizing higher maize yield and quality parameters as tangibly evident from the heatmap (Fig. 9).

Discussion

Diversifying the existing dominant RWCS towards viable alternative maize-based systems particularly the MWCS^{16,21,35}, the conservation agriculture based CETMs (PRBZT–PRBZT; FBZT–FBZT) along with the appropriate P-fertilization practices (PFPs), could enhance and stabilize the yields besides improving soil health in long-run^{17,63}, and more importantly the quality parameters of this potential food and industrial crop of south-Asia. The tillage and input-intensive RWCS in the IGPR of south-Asia is facing multiple production- and resource-vulnerabilities viz. exaggerating decline in crop productivity, groundwater table, input-use efficiencies and soil-health^{5,11,17,21}. Henceforth, CA based MWCS has ample potential to combat these assailabilities besides resolving twin challenges of maize productivity and quality enhancement for ushering in food and nutritional security vis-à-vis augmenting industrial applications of this crop in south-Asia.

In this study, the CA-based double zero-tilled permanent raised-bed system (PRBZT–PRBZT) with crop residue retention at 6 t ha⁻¹ per year in MWCS had significantly ($p < 0.05$) higher maize grain yield by 13.1% over the CT-based FBZT–FBZT, and by 5.7–6.4% over the double zero-tilled flat-bed system (FBZT–FBZT) and the single crop basis zero-tilled system in preceding wheat (RBCT–RBZT) across the years (Fig. 1). It could be associated with the positive impact of crop residue retention and zero-tillage on modulation of soil temperature^{64,65}, improved water retention, infiltration and moisture conservation^{29,66}, soil surface characteristics⁵, reduced

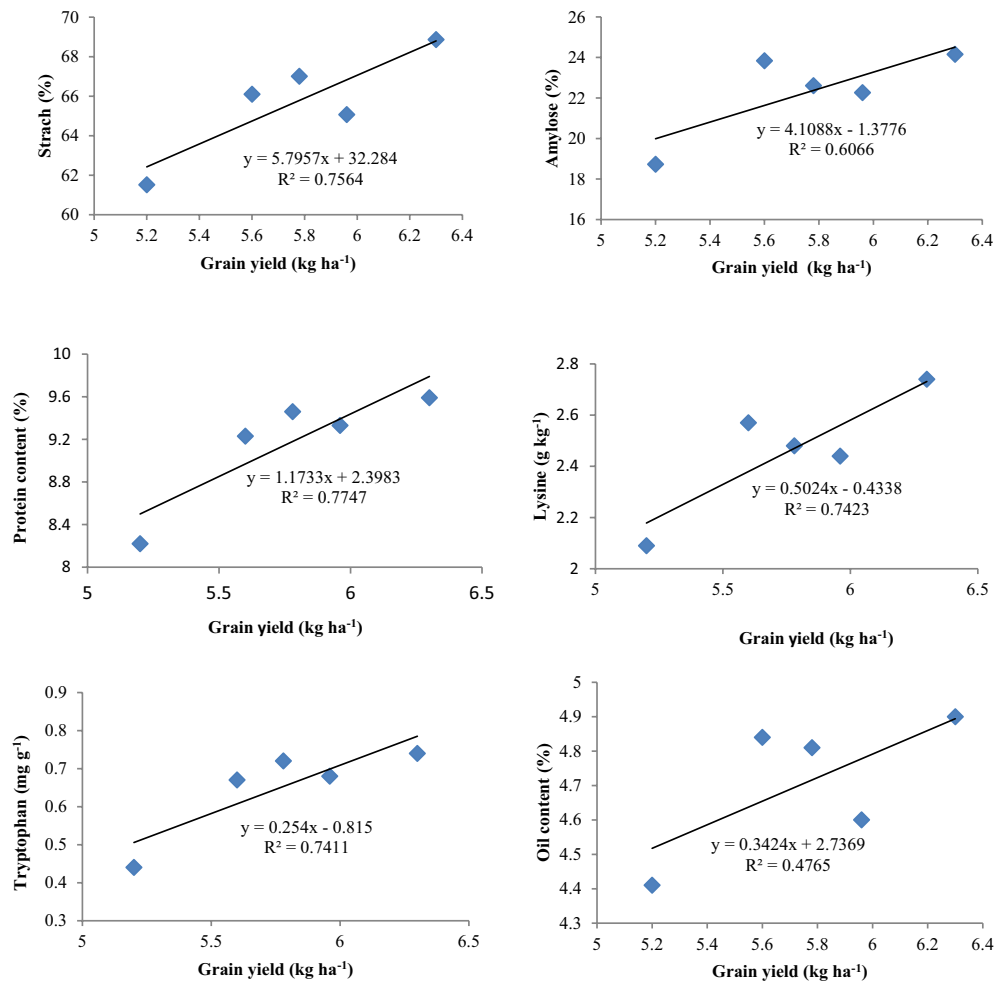


Figure 6. Correlation between grain yield and quality parameters of maize under P-fertilization practices in maize under MWCS.

crop-weed completion^{67,68}, improved soil physico-chemical and biological properties^{20,34,69} and better water and nutrient usage^{17,27,30}, resulting in better plant growth and yield¹¹. Double ZT system provides better soil physical conditions due to less machine trafficking⁶⁵, better seed germination and optimal seedling establishment due to avoidance of hard crust formation on soil surface, a characteristic feature of alluvial soils of IGPR⁷⁰. Higher maize yield under PRB/RB plots (PRBZT-PRBZT, RBCT-RBZT) over the flat-bed CT and ZT plots may also be attributed to better root aeration and root anchorage in raised-beds^{7,71}, least water stagnation during rains²⁷, and better moisture conservation in rainless spans⁷². Crop residue retention and its slow decomposition enhance the SOC⁶ and soil moisture content²⁷, both of which are ideal for favorable soil biological activities in ZT^{20,73,74}, which eventually augment the nutrient bio-availability^{10,75} favoring growth and productivity^{67,70,76}.

Since, P-fertilization directly influences the root growth and development which in turn improved the vegetative and reproductive growth vis-à-vis maize yield^{57,77}. Integration of P₅₀ + PSB + AMF + 2FSP, a combination of soil applied-P, microbial inoculants (PSB, AMF) and the two foliar-P sprays (2% DAP), had significantly ($p < 0.05$) higher grain yield by 11.3–17.5% over the soil applied-P₁₀₀ and P₀ (Fig. 1). Furthermore, the integrated use of P₅₀ + PSB + AMF + 2FSP saved ~ 34.7% fertilizer-P over the soil applied-P₁₀₀ both in maize alone and on cropping system basis in MWCS. In alkaline soils of semi-arid IGPR, soil applied-P reacts with calcium (Ca) and magnesium (Mg) ions to form Ca and Mg phosphates making P unavailable to plants⁵⁷. Hence, 2 foliar-P sprays at knee-high and pre-tasseling stage of maize proved beneficial for P-absorption through foliage which enhanced the plant growth and photosynthetic activity leading to improved maize yield³⁸. Foliar-P skip the P-fixation and leads to higher PUE⁶², which otherwise is an unavoidable fate of soil applied-P in alkaline and acidic soils⁶¹. Inoculation of maize grains with PSB and AMF along with 50% soil applied-P proved effective even over 100% soil applied-P due to improved P-availability and uptake owing to their synergistic effect on P-solubilization and mobilization of fixed native- and applied-P^{51,78}. Furthermore, the AMF mycelia growth greatly enhances the root exploratory area (10–1000 folds), thus, helping in better nutrient and water acquisition^{61,79}. Exudation of organic acids/chelating agents by AMF mineralizes the organic residues and manures to release inorganic nutrients with better phyto-availability^{65,80} besides enriching soil microbial diversity^{7,8}, thus adding to better yields.

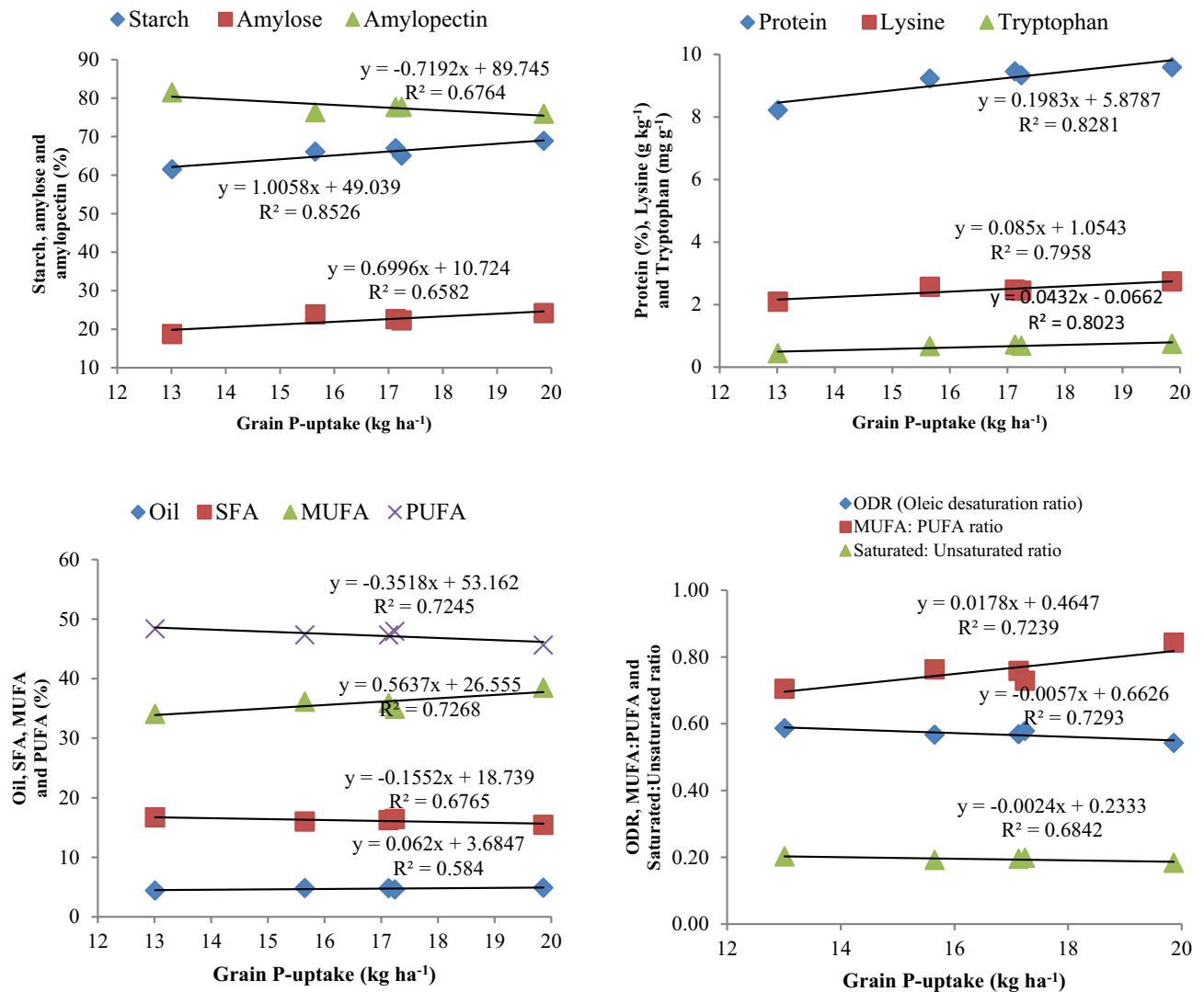


Figure 7. Correlation between grain P-uptake and quality parameters of maize under P-fertilization practices in maize under MWCS.

Phosphorus and potassium nutrition is vital for starch biosynthesis^{81,82}. The CA based CETMs (PRBZT-PRBZT, FBZT-FBZT, RBCT-RBZT) had higher starch content (65.1–67.2%) compared to 64.4% in the CT based FBCT-FBCT (Table 1). This may be attributed to enhanced macro- and micronutrient availability especially P and K³⁰, owing to residue decomposition with better nutrient-recycling especially K^{10,83}, and mineralization and solubilization of native- and applied-P by the organic acids released from decomposing residues under ZT^{28,84}. Likewise, maize under raised-beds (PRBZT-PRBZT, RBCT-RBZT) exhibited higher starch content (~66.2%) compared to ~64.9% under flat-beds (FBZT-FBZT, FBCT-FBCT) irrespective of tillage practices owing to better root aeration and anchorage for nutrients⁷¹ especially limiting nutrients like P⁶¹. Thus, ZT based CETMs enhanced the starch content over the conventional-tillage. Among PFPs, significantly ($p < 0.05$) higher starch (68.9%) was obtained by integrated use of P₅₀ + PSB + AMF + 2FSP followed by P₅₀ + 2FSP and least under P₀. Here, foliar-P fertilization proved beneficial in higher P absorption by maize foliage and its assimilation which enhanced the starch content. Under P-deficiency, starch content decreases because of reduced ATP production in chloroplast resulting in reduced activity of ADPG enzyme, a key enzyme in starch metabolism; so the starch produced in chloroplast was unable to diffuse to cytoplasm as tri-phosphate, thus, resulting in reduced translocation of carbohydrates to grains⁸². Starch content showed positive correlation with grain yield both for CETMs and PFPs, owing to greater role of starch in grain biomass accumulation being influenced by both CETMs³⁰, and P-fertilization³⁸. The CETM practices again did not show any significant effect on amylose and amylopectin content in maize grains like starch content. Starch biosynthesis is mainly dependant on proteins present in the starch granules⁸², particularly the granule-bound starch synthase I protein (GBSSI) which is involved in amylose synthesis⁸⁵. Hence, enhanced N-availability and protein content in ZT based CETMs might have enhanced the amylose content to some extent over CT based CETMs. Henceforth, higher amylose content under PRBZT-PRBZT and P₅₀ + PSB + AMF + 2FSP may be associated to improved nutrient availability and acquisition^{86–88}. Higher amylose content under PRBZT-PRBZT and 50% P + PSB + AMF + 2FSP, considered as a beneficial trait due to its lower glycemic-index, required by diabetic patients⁴⁵. As, amylose

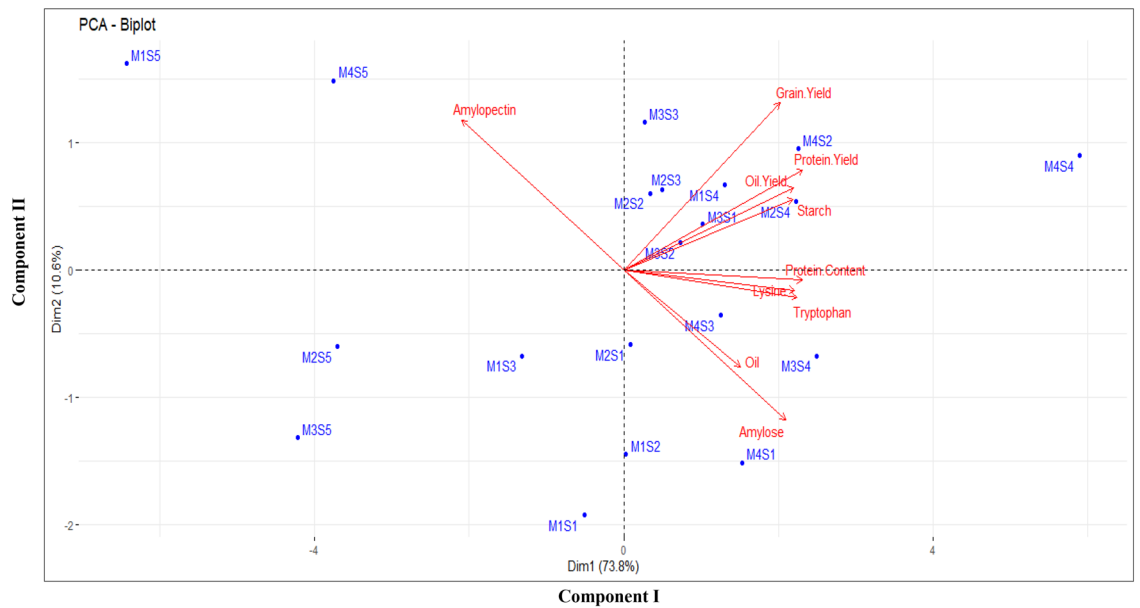


Figure 8. PCA biplots showing the effect of different treatment combinations of CETMs and PFPs on productivity and quality parameters of maize (pooled data).

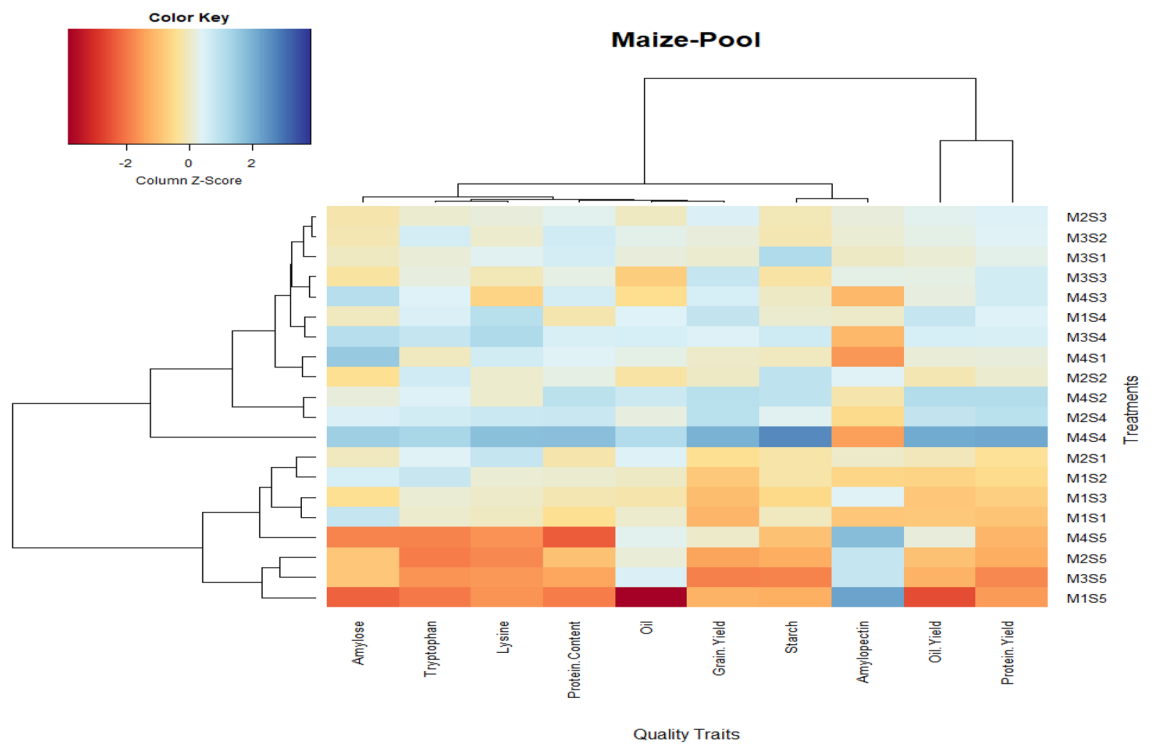


Figure 9. Biclustering heatmap analysis of maize productivity and the quality parameters at different treatment combinations of CETMs and PFPs in maize (pooled data), using R-software package ‘gplots’, Software version number ‘R package version 3.1.1’, Software URL <https://CRAN.R-project.org/package=gplots>.

and amylopectin together constitute the starch, hence, the treatments having higher amylose had a lower amylopectin content and vice-versa as evident from correlation studies and the heatmap clustering. Furthermore, amylose content and grain yield had positive correlation while amylopectin had negative correlation with yield both under CETMs and PFPs. Starch yield was significantly influenced by CETMs and PFPs due to higher grain yield under PRBZT–PRBZT and $P_{50} + PSB + AMF + 2FSP$ treatments. In nutshell, PRBZT–PRBZT along with $P_{50} + PSB + AMF + 2FSP$ proved beneficial to harness higher starch content, starch yield and amylose content; which can amplify the maize based food, starch and pharmaceutical industry in the south-Asia.

The ZT based CETMs had higher protein content over the conventional-tillage due to residue retention (3–6 t ha⁻¹ per year) which on decomposition and mineralization enhanced the N-availability and uptake to synthesize amino acids and proteins^{20,89,90}. Higher protein content in maize may also be associated with preferential deposition of *zein* protein over other endosperm proteins^{89,91}. Protein yield was higher under ZT based CETMs compared to CT plots due to improved nutrient availability and soil health^{92,93}, optimal soil moisture status and better root activities^{88,94}. The PFPs had significant influence on protein content and protein yield owing to the vital role of P in protein biosynthesis and energy relations^{81,82}. Furthermore, the P and N are found to have synergistic effect, thus, integrated use of P₅₀ + PSB + AMF + 2FSP might have significantly enhanced the N uptake and assimilation⁹⁵, leading to greater protein content and protein yield⁹⁶. Nutritionally essential amino acids viz. lysine and tryptophan are highly important to improve maize grain quality⁹⁷. Here, different CETMs had non-significant effect on lysine and tryptophan content. It may be strengthened with the fact that an increase in grain-N content as a result of improved N-availability is accompanied by decrease in the relative lysine content of grain proteins⁹⁸. On the other hand, P indirectly influences the lysine content because when P-supply is reduced, it results in reduced grain yield but with increased grain-N concentration; thus, leads to reduced lysine content in grains⁹⁸. Although under optimal P-fertilization, here P₅₀ + PSB + AMF + 2FSP, the grain yield increases which results in reduced grain-N content due to dilution effect, which in turn, increases the lysine content under optimal or excess P-supply⁹⁹. Henceforth, a similar pattern was observed for lysine content under PFPs in current study. Since, P and Zn are found to have antagonistic effects, so the P plays a vital role in tryptophan production¹⁰⁰. The Zn is involved in various oxidation–reduction reactions¹⁰¹; thereby, Zn-deficiency leads to oxidation of auxins and reduction of tryptophan¹⁰². As, tryptophan is the precursor of auxins⁹⁷, hence, tryptophan was higher under P₅₀ + PSB + AMF + 2FSP over soil applied-P₁₀₀ because of reduced Zn-uptake under soil applied-P₁₀₀ compared to P₅₀ + PSB + AMF + 2FSP, a combination of soil, microbial and foliar-P application which had an advantage over soil applied-P owing to reduced competition for Zn-uptake^{100,103}. Positive correlation of protein, lysine and tryptophan content with the grain yield both under CETMs and PFPs, further emphasize the importance of ZT based CETMs and integrated use of P₅₀ + PSB + AMF + 2FSP in enhancement of protein, lysine and tryptophan content as well as protein yield. Thus, deployment of such CPTs may altogether boost farm productivity and quality for better profitability of resource-poor south-Asian farmers and the maize based food, feed, and pharmaceutical industry in the region.

Different CETM practices had non-significant effect on oil content in maize grains although ZT based CETMs proved superior over conventional-tillage; where PRBZT–PRBZT had higher oil content due to crop residues decomposition¹⁰⁴, which slowly released the essential nutrients (soluble-P and S-compounds) into rhizosphere which later became available to plants specifically during reproductive phase¹⁰⁵. Sulfur (S) is a key element in chlorophyll formation, yield enhancement and oil synthesis⁵⁷. Furthermore, S-concentration and S-uptake has a strong synergistic relationship with P in plants^{57,106}. Positive correlation and heatmap clustering between oil content and grain P uptake under PFPs has further strengthens this fact. As per an estimate, the wheat straw of 2700 kg ha⁻¹, on average add ~ 28 kg N, 4.5 kg P, 52 kg K and 6 kg S ha⁻¹ under ZT system; on the other hand, this advantage may lack in CT system¹⁰⁷. Hence, crop residues decomposition released both P and S while additional application of foliar-P augmented S uptake from the soil, thereby, enhancing the oil content. The P₅₀ + PSB + AMF + 2FSP exhibited significantly higher oil content and oil yield over other PFPs which may be accrued to the fact that P directly participates in synthesis of oils, fats and phospholipids¹⁰⁸, besides its vital role in S acquisition⁵⁷. Better plant nutrition under CA based CETMs and integrated P-fertilization practices though caused a slight improvement in grain oil content in current study¹⁰⁹, but harnessed greater oil production per unit area because of enhanced grain yield³⁸. Positive correlation between oil content and maize grain yield has strongly established this relationship in current study. Hence, maize cultivation under PRBZT–PRBZT system along with P₅₀ + PSB + AMF + 2FSP may lead to higher corn-oil productivity which may cut down the oil imports by the developing nations like India.

The ZT and CT based CETMs exhibited non-significant effect on the composition of fatty acids viz. saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acid (PUFA) like oil content. However, these fatty acids were greatly influenced by PFPs both under CT and ZT systems with pattern of fatty acid composition as PUFA > MUFA > SFA both under CETMs and PFPs¹¹⁰. With increase in P-supply, SFA (Palmitic acid + Stearic acid) content decreased while MUFA (Oleic acid) content increased considerably, again as an indicator of better oil quality⁴². These observations are in agreement with the findings of Ray³⁸, who observed similar findings with respect to SFA and MUFA content while using higher doses of plant nutrients. The PUFA (linoleic acid) content were higher under P₀ in current study; which further corroborate with the findings of Krueger⁵⁶, who observed an increase in linoleic acid with the P-omission. In current study, the influence of different CETMs and PFPs on the status of individual fatty acid may not lead to definite conclusion about the overall fatty acid composition in corn-oil. Thus, various fatty acid ratios were estimated to draw logical conclusions. Among CETMs, double zero-tilled PRBZT–PRBZT had significantly higher MUFA: PUFA ratio and P/S index over CT plots which show better oil quality under ZT system owing to better N-supply encouraging carbon chain elongation in linoleic acid (PUFA) and oleic acid (MUFA)^{111–113}. The P/S index is a vital factor among all parameters as it represents the nutritional value of edible oils³⁸. Here, P/S index was found to be > 1.0 irrespective of CETMs and PFPs, which sufficiently indicated the better nutritional value of corn-oil with reduced tendency of deposition of lipids in the human body¹¹⁴. It was found that ZT based CETMs and P₅₀ + PSB + AMF + 2FSP exhibited higher P/S index over the CT system and P₀. It is reported that with an increase in unsaturation content and a degree in fatty acid, the susceptibility to oil oxidation increases; thus, releasing free radicals causing off-flavor and reduced nutritional quality³⁸. On average, oleic acid is 25-times less vulnerable to oxidation compared to linoleic acid, while linoleic acid is 2-times less susceptible compared to linolenic acid because of an increase in bond association energy as compared to linolenic acid¹¹⁵. As, ZT based CETMs and P₅₀ + PSB + AMF + 2FSP had higher oleic acid and lowest linoleic acid content, a positive sign for producing good quality corn-oil having

less susceptibility to oxidation which may help in flourishing the corn-oil industry. Linolenic acid is susceptible to oxidation and causes adverse effect on human health like cardiovascular diseases and improper brain development^{116,117}. As corn-oil contained negligible amount (< 1%) of linolenic acid in current study, hence, it would not exert any adverse effect on human health³⁸. Under P₀, higher oleic desaturation ratio (ODR) and lesser MUFA: PUFA ratio compared to other PFPs again point out a better quality corn-oil¹⁰⁵. Higher ODR indicates better and longer shelf-life of corn-oil; while lower ODR inhibits the subsequent desaturation steps which lead to reduced linolenic acid content¹¹². The P-fertilization considerably increased the ODR and MUFA: PUFA ratio under P₅₀ + 2FSP, P₅₀ + PSB + AMF and sole P₁₀₀; but P₅₀ + PSB + AMF + 2FSP showed slight reduction in ODR and an increase in MUFA: PUFA ratio may be due to enhanced P-availability over P₀. The MUFA: PUFA ratio is directly linked with the oxidative stability and nutritional properties of the oil¹¹⁸, thus, indicating that optimal P-nutrition and the ZT system both may improve the oil quality due to sustained and synchronized P bio-availability throughout crop season. Significantly higher P/S index under P₅₀ + PSB + AMF + 2FSP is another indicator of better nutritional value of edible maize oil³⁸. The heatmap also demonstrated that optimal P-nutrition and ZT system proved highly promising in producing good quality corn-oil, a good indication for corn-oil industry to target health conscious clientele¹¹⁴.

The CETMs and PFPs showed significant influence on P uptake in maize grains with greater magnitude under PRBZT-PRBZT and P₅₀ + PSB + AMF + 2FSP, owing to higher grain-P concentrations and maize yield in these treatments. Higher grain-P uptake in PRBZT-PRBZT is attributed to affirmative effects of crop residue retention which added substantial amount of nutrients including P in soil while improving soil physico-chemical and microbiological properties compared to CT plots^{27,72,119}. The P-fertilization along with PSB and AMF vis-à-vis foliar-P had a significant influence on grain-P uptake as a result of optimal P bio-availability⁶¹, better root and shoot system¹¹, enhanced native and applied-P solubilization and mobilization^{65,120}, and foliar-P supplementation⁵⁸; which collectively led to higher P uptake¹²¹. Thus, better the P-fertilization better is the P uptake by the crop and its subsequent accumulation in grains⁶². The P-fertilization in adequate amounts is essential for root and shoot development, seed formation and biochemical reactions viz., synthesis of proteins, oils and fats, phospholipids and energy relations, thus, it played a vital role in enhancing the maize quality¹⁰⁸. That's why, the quality parameters of maize viz., starch, protein, lysine, tryptophan, MUFA, and MUFA: PUFA ratio had positive correlation with grain-P uptake. Contrary to that, the amylopectin, PUFA, SFA, ODR and SFA: UFA ratio showed an inverse relationship with the grain-P uptake owing to complex interrelationships with their counterpart constituents^{82,85,108}, and with varying P supplies as reported by various researchers^{86–88,99,100}. The heatmap biclustering validated the superiority of CA-based PRBZT-PRBZT and FBZT-FBZT systems in combination with two PFPs viz. P₅₀ + PSB + AMF + 2FSP and P₅₀ + PSB + AMF in enhancing the grain, protein and oil yield as well as starch, amylose, lysine and tryptophan content; which demonstrate the sustainability of CA based crop management over the conventional agriculture while integrating P₅₀ + PSB + AMF + 2FSP in MWCS. It is tangibly evident from the PCA analysis and heatmap biclustering that the M₄S₄, a combination of double zero-tilled PRBZT-PRBZT system in combination with P₅₀ + PSB + AMF + 2FSP may prove highly sustainable for realizing higher maize grain yield and quality under a maize-wheat cropping system in a semi-arid agro-ecology. Thus, clean production technologies like double zero-tilled PRBZT-PRBZT along with P₅₀ + PSB + AMF + 2FSP not only enhanced the maize yield significantly while saving ~ 34.7% fertilizer-P both in maize and MWCS, but they also augmented the maize quality parameters to reinforce the food and nutritional security besides boosting food, corn-oil and starch industry in the south-Asia.

Conclusions

In order to safeguard the food and nutritional security of millions of south-Asian families concurrently conserving the soil, environment and natural resources, the application of clean production technologies (CPTs) like CA-based CETMs (PRBZT-PRBZT/FBZT-FBZT) that allows rapidly increases of yield and food quality should be a norm, not the exception. In our study, the production technology of the PRBZT-PRBZT/FBZT-FBZT along with integrated use of P₅₀ + PSB + AMF + 2FSP in MWCS proved to be excelled in the maize yield and quality parameters. On average, double zero-tilled PRBZT-PRBZT system and P₅₀ + PSB + AMF + 2FSP both significantly enhanced the maize grain, starch, protein and oil yield by 13.1–19% and 12.5–17.2%, over their respective counterpart treatments i.e. FBCT-FBCT and 100% soil applied-P (P₁₀₀); while concurrently saving ~ 34.7% fertilizer-P both in maize (20.8 kg P₂O₅/ha) and on cropping system basis (41.6 kg P₂O₅/ha). Integrated use of P₅₀ + PSB + AMF + 2FSP had significantly higher starch, amylose, protein, lysine, tryptophan and oil content by 1.2–10.4% over the 100% soil applied-P due to sustained and synchronized P bio-availability to the crop. The PRBZT-PRBZT had greater MUFA (oleic acid, 37.1%), MUFA: PUFA ratio (0.79) and P/S index (3.09). The ODR, MUFA: PUFA ratio and P/S index responded positively and significantly to P-fertilization practices. Double zero-tilled PRBZT-PRBZT system concurred with residue retention at 6 t ha⁻¹ per year along with P₅₀ + PSB + AMF + 2FSP while saving ~ 34.7% fertilizer-P in MWCS, proved as a potential clean production technology for enhancing the maize productivity and quality. Accordingly, deserves strong recommendation to augment maize yield and quality besides augmenting safe industrial uses in maize based industries, climate-resilience and farmers' well-being in semi-arid IGPR in south-Asia and similar agro-ecologies across the globe.

Materials and methods

Experimental details and crop management. A field experiment was carried-out in maize (*Zea mays* L.) for two years during *Kharif* 2018 and 2019 at Experimental Farm of ICAR-Indian Agricultural Research Institute, New Delhi, India [Latitude 28° 63' N; Longitude 77° 15' E; Altitude 228.6 m] under maize-wheat cropping system (MWCS). This experimental site is located in semi-arid sub-tropics having sandy-loam Alluvial soil

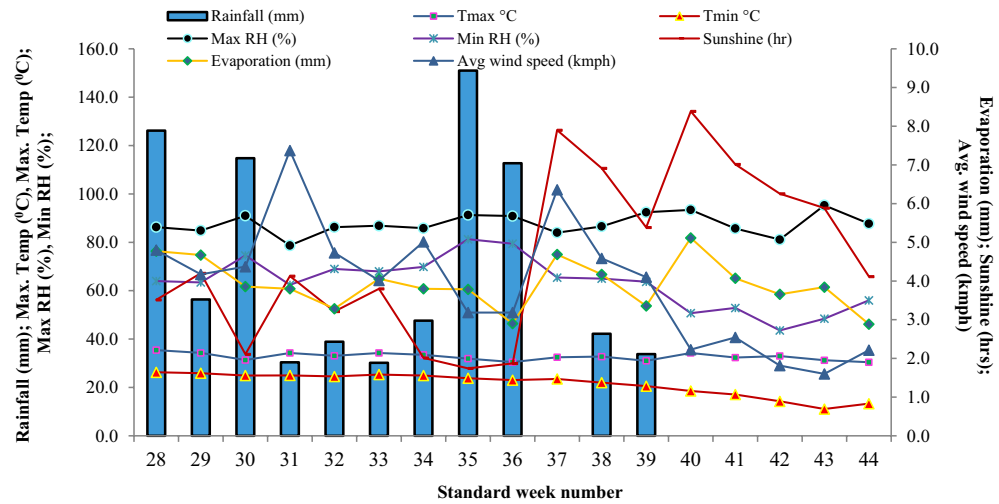


Figure 10. Weekly weather conditions during the cropping period of *Kharif* season maize in the Indo-Gangetic Plains, 2018.

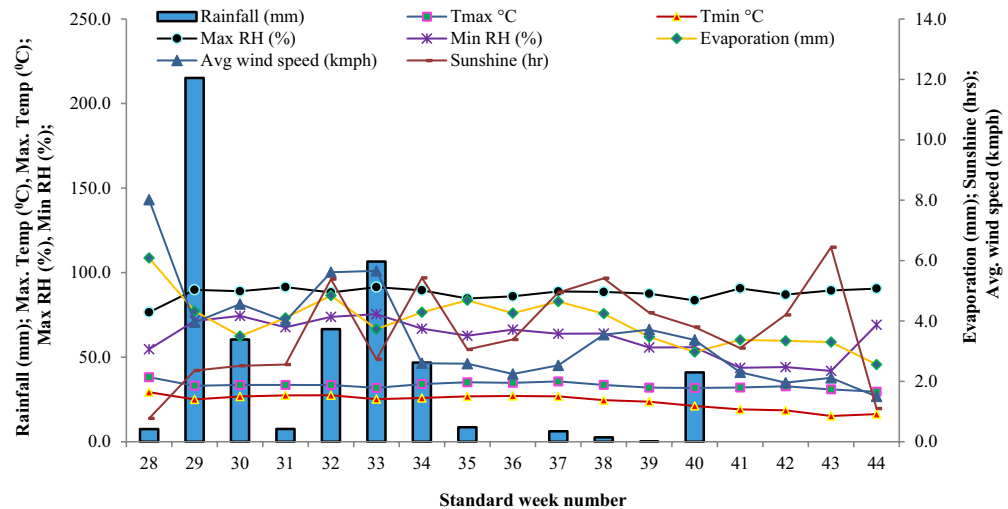


Figure 11. Weekly weather conditions during the cropping period of *Kharif* season maize in the Indo-Gangetic Plains, 2019.

belonging to *Typic Ustochrepts*. Climate is semi-arid with dry hot summers and cold winters with May and June as hottest months with mean daily maximum temperature varying from 40–46 °C (Figs. 10, 11).

Average annual rainfall is ~ 650 mm 80% of which is received through 'South-West Monsoons' during July–September and the rest during 'Western Disturbances' from December to February. Mean annual evaporation is ~ 850 mm. Physico-chemical analysis of composite soil samples (0–15 cm depth) was done at the start of the experiment using standard procedures (Table 4). Soil had pH 8.0, oxidizable soil organic-C 0.421%, alkaline KMnO_4 oxidizable-N 137.9 kg ha⁻¹, 0.5 M NaHCO_3 extractable-P 12.9 kg ha⁻¹ and 1 N NH_4OAc extractable-K 302.8 kg ha⁻¹.

The experiment was laid-out in split-plot design with 3-replications and 20-treatment combinations comprised of 4 main-plot treatments i.e. crop establishment and tillage management (CETM) practices [M_1 : FBCT–FBCT (Flat bed–conventional tillage both in maize and wheat); M_2 : RBCT–RBZT (Raised bed–conventional tillage in maize and raised bed–zero tillage in wheat); M_3 : FBZT–FBZT (Flat bed–zero tillage both in maize and wheat); M_4 : PRBZT–PRBZT (Permanent raised bed–zero tillage both in maize and wheat)], and 5 P-fertilization practices in sub-plots [S_1 : P_{100} (100% P as basal); S_2 : P_{50} + 2FSP {50% P as basal (P_{50}) + 2 foliar sprays of phosphorus (2FSP) as DAP (2%) at knee-high stage (KHS) and pre-tasseling stage (PTS) in maize and at tillering stage (TS) and pre-flowering stage (PFS) in wheat}; S_3 : P_{50} + PSB + AMF { P_{50} + PSB + AM-fungi (AMF)}; S_4 : P_{50} + PSB + AMF + 2 FSP (P_{50} + PSB + AMF + 2FSP at KHS and PTS in maize, and at TS and PFS in wheat); S_5 : P_0 {100% N and K with no-P (P_0) as control}]. Crop residues of preceding season wheat and maize crops were applied at 3 t ha⁻¹ to all the ZT-plots except CT-plots after sowing of the succeeding crops of maize

S. No.	Particulars	Values	Method followed
1	Mechanical analysis		
	Sand (%)	64.9	Hydrometer method ¹²²
	Silt (%)	21.0	
	Clay (%)	14.1	
	Textural class	Sandy-loam	
2	Physical properties		
	Bulk density (Mg m ⁻³)	1.57	Veihmeyer and Hendrickson ¹²³
3	Chemical properties		
	Organic carbon (%)	0.42	Walkley and Black method ¹²⁴
	Available N (kg ha ⁻¹)	137.9	Alkaline permanganate method ¹²⁵
	Available P (kg ha ⁻¹)	12.9	Olsen's method ¹²⁶
	Available K (kg ha ⁻¹)	302.8	Flame photometer method ¹²⁴
	pH (1:2.5 soil: water ratio)	8.0	Beckman's pH meter ¹²⁷
	EC (dSm ⁻¹) (1:2 soil: water ratio)	0.46	Richards ¹²⁸
4	Biological properties		
	Soil microbial biomass carbon (µg SMBC g soil ⁻¹)	183.4	Nunan et al. ¹²⁹
	Dehydrogenase activity (µg TPF g soil ⁻¹ day ⁻¹)	28.3	Casida et al. ¹³⁰
	Alkaline phosphatase activity (µg PNP g soil ⁻¹ h ⁻¹)	185.4	Tabatabai and Bremner ¹³¹
	Acid phosphatase activity (µg PNP g soil ⁻¹ h ⁻¹)	29.2	Tabatabai and Bremner ¹³¹

Table 4. Initial fertility status of the experimental site.

and wheat, respectively. In current study, 'PMH-1' high yielding single cross maize hybrid was used as the test cultivar being one of the most promising and popular cultivar of Indian IGPR. Hybrid 'PMH-1' was sown in gross plot size of 5.0 × 4.2 m at plant spacing of 60 × 25 cm using seed drill with seed at 20 kg ha⁻¹ and fertilizer recommendation of N: P₂O₅: K₂O at 150: 60: 40 kg ha⁻¹ on 12th and 9th July and harvested on 29th and 24th October during *Kharif* 2018 and 2019, respectively. Whole K and whole treatment-wise fertilizer-P were applied as basal dose while N was applied in 3 equal splits (1/3rd as basal, 1/3rd top-dressed at KHS, 1/3rd top-dressed at PTS). Foliar P-fertilization was done at KHS and PTS using 2% DAP (Di-ammonium phosphate; 18% N and 46% P₂O₅) in 750 L water ha⁻¹. Except treatments, maize crop was grown using standard crop management practices¹³².

Maize grain yield, protein content and protein yield. After harvesting, the maize crop from net-plots was sun-dried, threshed plot-wise, grains cleaned and sun-dried till 10% seed moisture was obtained. Grain yield (t ha⁻¹) was estimated using standard procedures¹³². Nitrogen content (%) in maize grains was determined using standard procedure¹³². Protein content (%) in maize grains was calculated by multiplying grain-N content (%) by the factor 6.25 while protein yield (kg ha⁻¹) in maize grains was calculated by using following formula:

$$\text{Protein yield (kg/ha)} = \left(\frac{\text{Protein content (\%)} \times \text{Grain yield (kg/ha)}}{100} \right)$$

Starch estimation. A grain sample of 0.4 g was homogenized in hot 80% ethanol to remove sugars. The residues retained after centrifugation were washed repeatedly with hot ethanol (80%) till the washing is colorless. The residues were dried and the extraction was done from the dried samples with the application of 5 mL water and 6.5 mL of perchloric acid (52%). The 0° C temperature was maintained for 20 min (min) and then samples were put under centrifugation at 10,000 rpm for 8 min. The supernatant was decanted and kept for starch estimation. The extraction was repeated 2–3 times for full and final extraction. With the addition of distilled water, final volume of the pooled-up supernatant was made to 100 mL. The 0.1 mL of supernatant was pipetted-out and the volume was made-up to 1.0 mL with distilled water. Similarly for reference, different aliquots of standard glucose solution were taken and volume was made-up to 1.0 mL using distilled water. The 4.0 mL of anthrone reagent was added to each tube and heated for 8 min in water bath. Intensity of color, green to dark green, was recorded at 630 nm¹³³. The glucose concentration of the samples was determined using the calibration curve and the values obtained were multiplied by a factor 0.9 to quantify the starch content (%). Starch yield (kg ha⁻¹) in maize grains was calculated by using following formula:

$$\text{Starch yield (kg/ha)} = \left(\frac{\text{Starch content (\%)} \times \text{Grain yield (kg/ha)}}{100} \right)$$

Amylose and amylopectin content. Maize grains from different plots were ground to make fine powder with particle size of 500 µ after milling. 100 mg of powdered samples was added with a mixture of ethanol and 1 M NaOH (1 mL + 10 mL) and was left as such overnight. Subsequently, distilled water was added to sample

solution to make the final volume to 100 mL. An aliquot of 2.5 mL of extract was mixed with 20 mL distilled water and 3 drops of Phenolphthalein, where by the solution changes into pink-color. On addition of 0.1 M HCl drop by drop, the pink color disappears. To the treated sample, 1 mL of iodine reagent was added and volume was made-up to 50 mL by adding distilled water and then absorbance was recorded at 590 nm with reference to blank (1 mL iodine reagent diluted to 50 mL with distilled water). The amylose content in maize grains was determined using standard curve derived from potato amylose¹³⁴. Standard amylose solution was prepared by dissolving 100 mg in 10 mL of 1 M NaOH and making up to 100 mL final volume. The amount of amylose in samples was determined by using standard curve prepared from amylose (0.2, 0.4, 0.6, 0.8 and 1.0 mL) against a blank for which dilute 1 mL of iodine reagent to 50 mL with water. The relevant calculations were done using following formula:

$$\text{Amylose content} = \left(\text{O.D.} \times \frac{\text{Dilution factor}}{\text{Slope}} \right)$$

Since, 2.5 mL of the test solution = x mg amylose; therefore, 100 mL contains = $\left(\frac{x}{2.5} \times 100 \right)$.

The amylopectin content (%) in maize grains was determined by subtracting the amylose content from the total starch content¹³⁵.

Lysine and tryptophan estimation. The 5 mL papain solution was added to 100 g defatted maize grain sample and incubated at 65 °C overnight. It was cooled down to room temperature, centrifuged and decanted. Carbonate buffer (0.5 mL, pH 9.0) and copper phosphate suspension (0.5 mL) was added to 1 mL digest; after that the mixture was shaken for 5 min in a vortex mix and centrifuged. To 1 mL supernatant 0.1 mL of pyridine reagent was added, mixed well and shaken for 2 h. Then after adding 5 mL of 1.2 M HCl and mixing, extraction was done 3 times with 5 mL ethyl acetate, and ethyl acetate top layer was discarded. The absorbance of aqueous layer was read at 390 nm¹³⁶. The standard lysine solution was prepared by dissolving 62.5 mg lysine mono hydrochloride in 50 mL carbonate buffer. For preparing a standard curve, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the standard lysine solution was pipetted out in different test tubes and final volume of 1 mL was made using carbonate buffer. Later, added 4 mL papain to each tube and mixed thoroughly. Now, 1 mL was pipetted out and 0.5 mL of amino acid mixture and 0.5 mL of copper phosphate suspension were added to it. Afterwards, 1 mL solution from each test tube was transferred to other test tubes and adding 0.5 mL amino acid mixture and 0.5 mL copper phosphate suspension to each one. The above steps were repeated as followed in case of samples and the absorbance of aqueous layer was read at 390 nm¹³⁶. The lysine content in maize samples was determined from standard curve and results were expressed as g kg⁻¹ dry matter.

For estimation of tryptophan, 15 mg defatted maize grain sample was taken in three different 50 mL conical flasks. In 2 flasks, 30 mg of *p*-dimethyl amino benzaldehyde was added. Third flask acted as the blank. To all the flasks, 9.5 M H₂SO₄ solution was added. The flasks were kept in dark for 20 h at 30 °C followed by addition of 0.1 mL of 0.045% NaNO₂ solution to each flask. After mixing, the flasks were again kept for 30 min at room temperature. After centrifugation, the absorbance of blue color of the solution was measured at 660 nm¹³⁷. A standard curve of tryptophan was prepared by taking various concentrations (10 to 60 µg mL) of standard tryptophan solution; the volume was made up to 0.6 mL by adding distilled water followed by addition of 9.4 mL of 9.5 M H₂SO₄ solution slowly and mixed gently. Same steps were followed for the standard solutions. Tryptophan content in the samples was determined from standard curve and expressed as µg g⁻¹.

$$\text{Tryptophan } (\mu\text{g/g}) = \frac{\mu\text{g tryptophan from standard curve}}{\text{Weight of grain sample (g)}}$$

Oil content and oil yield. Oil content (%) in maize grains was determined by petroleum ether extraction in a Soxhlet apparatus for 16 h according to AOAC procedure 948.22¹³⁸. Oil yield (kg ha⁻¹) in maize grains was calculated by using following formula:

$$\text{Oil yield (kg/ha)} = \left(\frac{\text{Oil content (\%)} \times \text{Grain yield (kg/ha)}}{100} \right)$$

Fatty acid analysis and fatty acid ratios. The 100 mg powdered maize grain samples were defatted with solvent mixture of Chloroform:Hexane:Methanol (8:5:2 v/v) for fatty acid analysis. The extracts were dried under a stream of nitrogen and fatty acids were converted into methyl-esters using 0.5 M KOH and 0.5 M HCl. Fatty acids were separated using Gas Chromatography-Mass Spectrometry (GC-MS) following the method as suggested by Kumar and Dhillon¹³⁹. Separation of fatty acids viz. Palmitic acid, Stearic acid, Oleic acid and Linoleic acid was carried-out using HP Innnowax capillary column (30 m × 0.32 mm × 0.5 µm). The separated peaks were identified on the basis of retention time of standard fatty acid peaks and confirmed using GC-MS library. Besides fatty acid synthesis, different fatty acid ratios viz. ODR, MUFA: PUFA, SFA: UFA and PUFA: SFA were also worked-out using standard formulae³⁸. These ratios were calculated excluding the linolenic acid because its contribution to total fatty acid composition was < 1% in maize grain oil.

$$\text{ODR} = \frac{\%C_{18:2}}{\%C_{18:1} + \%C_{18:2}}$$

$$\text{MUFA : PUFA} = \frac{\%C_{18:1}}{\%C_{18:2}}$$

$$\text{SFA : UFA} = \frac{\%C_{16:0} + \%C_{18:0}}{\%C_{18:1} + \%C_{18:2}}$$

The P/S index is the ratio of polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) and it was calculated by the following formula¹⁴⁰:

$$\text{P/S index} = \frac{\text{PUFA}}{\text{SFA}}$$

where ODR = Oleic desaturation ratio; MUFA = Monounsaturated fatty acid (Oleic acid); PUFA = Polyunsaturated fatty acid (Linoleic acid); SFA = Saturated fatty acid (Palmitic acid + Stearic acid); UFA = Unsaturated fatty acid; P/S index = PUFA/SFA ratio; C_{18:1} = Oleic acid; C_{18:2} = Linoleic acid; C_{16:0} = Palmitic acid; C_{18:0} = Stearic acid.

Phosphorus content and its uptake in maize grains. Concentration of P in maize grains was determined by using the Vanadomolybdo-phosphoric acid yellow colour method at 420 nm wavelength on a UV–VIS spectrophotometer. From P content (%) in plants, P uptake (kg ha⁻¹) was computed using the formula given below:

$$\text{P uptake in grains (kg ha}^{-1}\text{)} = [\% \text{ P in grains} \times \text{grain yield (kg ha}^{-1}\text{)}]$$

Statistical analysis. The data related to each parameter were analyzed as per the procedure of analysis of variance (ANOVA) to determine treatment effects through Tukey's honestly significant difference test as a post hoc mean separation test ($p < 0.05$) by using SAS 9.1 software (SAS Institute, Cary, NC). Tukey's procedure was used where ANOVA was found significant (Supplementary Tables S1 and S2). A two-dimensional heatmap with hierarchical clustering of treatment-by-traits was drawn using R-software package 'gplots' developed by Warnes et al.¹⁴¹. To reduce the complexity of relationship, a data reduction technique was performed using principal component analysis (PCA) implemented in the R package 'Factoextra' and 'FactoMineR', and thereby resulting PC scores were plotted^{142,143}.

Research involving plants. It is stated that the current experimental research on the plants comply with the relevant institutional, national, and international guidelines and legislation. It is also stated that the appropriate permissions has been taken wherever necessary, for collection of plant or seed specimens. It is also stated that the authors comply with the 'IUCN Policy Statement on Research Involving Species at Risk of Extinction' and the 'Convention on the Trade in Endangered Species of Wild Fauna and Flora'.

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Author contributions

H.M.N. conducted the research study, carried out the biochemical analysis, assembled the data, and wrote the manuscript. A.K.C. conceptualized, designed and conducted the research study, carried out the biochemical analysis, assembled the data, wrote, reviewed and edited the manuscript. S.K. carried out the biochemical analysis and edited the manuscript. A.D. designed the research study. V.K.S. did the interpretations. V.K.S. carried out the plant chemical analysis. V.T. carried out the biochemical analysis. M.K.D. carried out the biochemical analysis. S.S. carried out the biochemical analysis. V.K.D. wrote the manuscript. N.S.D. performed the statistical analysis. B.M. performed the statistical analysis. S.S. did the interpretations. S.P. carried out the plant chemical analysis. A.K. assembled the data and wrote the manuscript. S.K.R. prepared the figures and tables. G.G. wrote and edited the manuscript. P.V. carried out the biochemical analysis. A.K. reviewed the manuscript. S.G. prepared the figures and tables and edited the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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