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Impact of Resistant Starch Accumulation on Chemical Composition and Grain Quality of Maize Genotypes

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A B S T R A C T

The present study disclose the nutritional characteristics of high and low resistant starch (RS) containing maize (*Zea mays* L.) genotypes and determined in order to identify different correlations between them. A set of 5 high and 5 low RS containing selfed maize genotypes, mostly hybrids of public as well as private sector widely grown and analyzed for the carbohydrate profile (amylose, amylopectin and starch), protein, oil, ash and crude fiber content. The results revealed significant variation for amylose and amylopectin in both high and low RS containing maize genotypes. Amylose content showed highly significant positive correlation ($r = 0.546$ and 0.550, $p < 0.05$, high and low RS containing maize genotypes, respectively), whereas amylopectin showed highly significant negative correlation ($r = -0.546$ and -0.550, $p < 0.05$, high and low RS containing maize genotypes, respectively). Protein content was positively correlated with RS ($r = 0.321$[high RS] and 0.311[low RS], $p < 0.05$) and amylose ($r = 0.454$ [high RS] and 0.464 [low RS], $p < 0.05$) in both high and low RS containing maize genotypes. Oil and crude fiber content also showed positive correlation with RS and amylose content in both high and low RS containing maize genotypes, whereas the starch and ash content were not altered significantly in both high and low RS containing maize genotypes.

Introduction

Maize (*Zea mays* L.) is the third most important crop after wheat and rice, widely cultivated in tropics, sub-tropics and temperate regions to almost all the conditions of irrigated to semiarid of the world. Maize providing nutrients for humans and animals and serving as a basic raw material for the production of starch, oil and protein, alcoholic beverages, food sweeteners and, more recently, fuel (RoufShah *et al.*, 2016). Annual maize production is 1016.73 million metric tonnes, of which Asia alone produces 304.31 million metric tonnes (FAOSTAT, 2013). It is estimated that more than half of the increased demand for cereals as a whole will come from maize farmers and consumers (Yan *et al.*, 2011).

Carbohydrate is the main chemical constituent of maize grain about 60-70% (RoufShah *et* *al.,* 2016), in which starch alone share about 80% part of total carbohydrate. Starch is a reserve polysaccharide occurring in granular form in higher plants and provides 70-80% of the calories consumed by human's worldwide (Cereda and Jane, 2010). Resistant starch (RS), a non-digestible form of total starch of maize grain has received much attention for both its potential health benefits (similar to soluble fibre) and functional properties. RSs are not digested in the small intestine by enzymes but fermented in the large intestine. Maize starch is composed of two biochemical constituents namely amylose and amylopectin. Amylose (water soluble) is a linear polymer of glucose units joined with α 1-4 linkage, whereas amylopectin (water insoluble) is the branched polymer containing glucose subunits joined through α 1-4 as well as α 1-6 linkage. Normal maize starch consists of 25–30 % amylose, and the rest of being as amylopectin. A mutant, amylose-extender (ae) mutant, produces starch with a much larger amylose content and amylopectin with significantly longer branch-chains than the normal maize starch (Baba *et al.,* 1982). Variability has been reported pertaining to amylose to amylopectin ratio in the normal maize genotypes. A higher content of amylose lowers the digestibility of starch due to positive correlation between amylose content and formation of RS in maize grain (Sajilata *et al.,* 2006).

Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize. Maize is poor in terms of protein content (~ 9.5%) and also has low biological quality in relation to essential amino acids lysine and tryptophan. Several natural maize mutants conferring higher lysine and tryptophan levels were identified, but the recessiveopaque-2 (o2) mutation was found to be the most suitable for genetic manipulation in breeding programs. The breeding efforts led to the development of quality protein maize (QPM), maize high in lysine and tryptophan content with good agronomical performances. The protein content is a quantitative trait and study proved that there is a great number of pathways involved in the synthesis of protein in plants. Protein is an expensive but necessary constituent of both food and feed (Vivek *et al.,* 2008).

Maize grain contains an average FA composition of 11% palmitic (16:0), 2% stearic (18:0), 24.1% oleic (18:1), 61.9% linoleic (18:2), and 0.7% linolenic (18:3) acids (Weber *et al.,* 1987). Developing maize oil with different arrangements of altered FA compositions can be beneficial in various ways. For example, increasing the oleic acid content would enhance oil oxidative stability (White *et al.,* 2002) and provide a more healthful FA composition that could decrease coronary heart disease (Mattson *et al.,* 1985) On the other hand, increased saturated (palmitic and stearic) FA composition would allow for production of margarines without hydrogenation and the subsequent formation of undesirable trans FAs (Duvick *et al.,* 2006) Crude fibre was found to be the fourth largest component (~ 2.0%) present in maize grain after carbohydrate, protein and fat. Enyisi *et al.,* (2014), reported a fibre content in the range of 2.07 – 2.97, for maize variety grains in Nigeria. Beside this ash content (~ 1.4%) also play significant role in maize grain quality.

The objective of this study was to establish the quality components (carbohydrate profile, protein, oil, ash and crude fiber content) level of maize grain with respect to resistant starch.

Materials and Methods

Raw materials

The experimental material used in this study consisted of 10 maize (5 high and 5 low resistant starch) genotypes were grown widely in different part of country. The genotypes were grown in Randomized Block Design (RBD) with three replications. Selfed pollinated ears from each replication were harvested at maturity stage; seeds were shelled under shade and stored in dark at 4°C to prevent any loss of nutritional quality. The samples were oven-dried to reduce the moisture level in order to meet the accuracy of the results. Individual samples were ground into fine powder using a Cyclotech Mill (Model 1093, FOSS, Sweden), defatted using petroleum ether and finally kept in desiccators for analysis of various nutritional quality parameters. After harvest, samples were taken for chemical analysis.

Chemical analyses

Total starch, in a separate aliquot of the acetate solution, is similarly hydrolysed to D-glucose which is measured calorimetrically by glucose oxidase/peroxidise (Megazyme, 2007). Amylose content was also estimated using megazyme kit method (2007).The specific formation of amylopectin complexes with the lectin concanavalin A (Con A) offers an accurate approach to amylose measurement. Amylopectin content is calculated by subtracting the amylose from total starch content. Resistant starch was estimated by using megazyme kit (2008), whereby non-resistant starch was solubilised and hydrolyzed to D-glucosby treatment with pancreatic α-amylase and amyloglucosidase (AMG). The reaction is terminated by the addition of an equal volume of ethanol or industrial methylated spirits (IMS, denatured ethanol) and the RS is recovered as a pellet on centrifugation. This is then washed twice by suspension in aqueous IMS or ethanol (50% v/v), followed by centrifugation. Free liquid is removed by decantation. RS in the pellet is dissolved in 2 M KOH by vigorously stirring in an ice-water bath over a magnetic stirrer. This solution is neutralised with acetate buffer and the starch is quantitatively hydrolysed to glucose with AMG. D-Glucose is measured with glucose oxidase/peroxidase reagent (GOPOD) and this is a measure of the RS content of the sample.

Protein content was determined by available nitrogen in sample by Kjeldhal, method SR EN ISO 5983-1/2006.One gram sample was digested in 20 ml sulphuric acid, at 400ºC using copper sulfate and potassium sulfate as catalyst mixture. Digested sample was distilled using 33-35% NaOH. Nitrogen is converted to ammonia, which is distilled and titrated with 0.1 N HCl to estimate the protein content. Crude protein content was estimated using a conversion factor of 6.25 for corn. Crude fat was extracted with petroleum ether by a Soxhlet apparatus. The fat content is calculated from the difference between the initial sample weight and the weight of the dried residue after extraction. The results are expressed as percentage (%) of total fat (SR EN ISO 6492:2001). Ash contents of each sample was determined by the interaction of dried sample in an electric muffle furnace at 550°C until the residue obtained was of grey colour and calculated (SR EN ISO 2171:2010). Crude fiber was determinated by using SR EN ISO 6865:2002.

Statistical analysis

Descriptive statistic and analysis of variance (ANOVA), correlation between biochemical traits was done using Statistical Analysis Software (SAS 9.2 English). A Pearson Correlation Coefficient |r| among 80 maize hybrids was calculated by taking Prob> | r | under (Null Hypothesis) H0: Rho=0 by Statistical Analysis Software.

Results and Discussion

During this study we have evaluated the quality indices which are significant evaluating the quality parameters for maize. As shown by the data estimated from the polyfactorial variance, amylose, amylopectin, total starch, protein content, oil content, ash content and crude fiber content in 5 high resistant starch (RS) and 5 low RS maize genotypes.

Starch is, quantitatively, the most important carbohydrate in the diet of most humans and their principal source of dietary energy. Although starch usually accounts for 60% of energy intake in developing countries, but, its consumption for human food is continuously declining in western world where adults consumption ranges between 120 to 150 g of starch daily. Variations in the composition of cereal starch, in terms of the amylose to amylopectin ratio, are governed by the genome and their genetic potential to undergo mutations (Rahman *et al.,* 2007). Maize has been found to possess 72% (Chaudhary, 1983) and 68 to 73% (Balconi *et al.*, 2007) of starch. RS is a very helpful quality component of maize for human health; such as lowering the glycemic index and promoting colon health (Sajilata *et al.*, 2006), prevention of colonic cancer (Nugent, 2005; Sajilata *et al.*, 2006), as a prebiotic agent (Perera *et al.*, 2010), inhibition of fat accumulation(Sharma *et al.*, 2008) and absorption of minerals (Lopez *et al.,* 2001; Younes *et al.*, 1995).Starch is made of two components; Amylose (unbranched; water soluble) and Amylopectin (branched; water insoluble). In which amylose is helical polymer made of α-D-glucose with α (1→4) glycosidic linkage. Normal maize starch consists of 15-30% amylose, depending on the botanical origin, degree of maturity, growing condition, and the method used for determination (Chung *et al.*, 2009; Hasjim *et al.*, 2009) whereas high-amylose starch usually consists of more than 50% amylose (Campbell *et al.*, 2007 ; Li *et al.*, 2008). One of the maize mutants, amylose-extender (ae) mutant, produces starch with a much larger amylose-content and amylopectin with significantly longer branch-chains than the normal maize starch (Jane *et al.,* 1999; Kasemsuwan *et al.,* 1995; Shi and Seib, 1995; Takeda *et al.*, 1993; Yuan *et al.*, 1993). In the present study we observed that the high RS containing genotypes (ranging from 4.41 to 5.12%) accumulated high amylose contents ranges 35.92 to 44.42% (figure 1 A), whereas low RS containing genotypes (ranging 1.81 to 2.36%) accumulated lower amylose contents ranges 24.63 to 32.75% (figure 2 A).We also estimated the accumulation of amylopectin in both high and low RS genotypes. Interestingly, we observed the lower level (ranges from 55.58 to 64.08%) of amylopectin in high RS and higher level (ranges from 67.25 to 75.37%) (Figure 1 A) in low RS containing genotypes (figure 2 A and B). Statistically result was recorded as very significantly positive in the case of amylose and significantly negative in case of amylopectin (table 1 and 2). Amylose content was positively correlated to RS ($r = 0.550$, $p < 0.05$) and amylopectin content was negatively correlated with RS ($r = -0.550$, $p < 0.05$) in both high and low RS containing maize genotypes. These results are the agreement with findings of Sajilata *et al.*, (2006).A higher content of amylose lowers the digestibility of starch due to positive correlation between amylose content and formation of RS. The amylopectin is a much larger molecule than amylose; therefore, it has a much larger surface area per molecule than amylose which makes it a preferable substrate for amylolytic attack.

Sievert and Pomeranz (1989) also reported that peas with 33% of amylose showed the highest amounts of RS (10.5%) and potatoes with 20% of amylose showed the lowest amounts of RS (4.4%). We also estimated the total starch content in both high (figure 1 A) and low (figure 2 A) RS containing maize genotypes and found that the accumulation of total starch content was not affected by the accumulation of RS, amylose and amylopectin.

Table.1 Phenotypic correlations between grain quality traits of high Resistant starch (RS) maize genotypes

Traits	Resistant Starch %	Amylose %	Amylopectin %	Total starch%	Protein content %	Oil content %	Ash content %	Crude fiber content %
Resistant Starch %	1.000							
Amylose %	0.546*	1.000						
Amylopectin %	-0.546*	-1.000	1.000					
Total starch%	-0.108	0.075	-0.075	1.000				
Protein content %	0.321*	0.454*	-0.454*	0.042	1.000			
Oil content %	0.353*	0.314*	-0.314*	-0.066	0.087	1.000		
Ash content %	0.216	0.058	-0.058	-0.156	0.112	-0.231	1.000	
Crude fiber content %	0.359*	0.353*	-0.353*	0.184	-0.138	-0.203	-0.158	1.000

*Correlation is significant at the 0.05 level

Table.2 Phenotypic correlations between grain quality traits of low resistant starch (RS) maize genotypes

Traits	Resistant Starch %	Amylose %	Amylopectin %	Total starch%	Protein content %	Oil content %	Ash content %	Crude fiber content %
Resistant Starch %	1.000							
Amylose %	0.550*	1.000						
Amylopectin %	-0.550*	-1.000	1.000					
Total starch%	-0.112	0.073	-0.073	1.000				
Protein content %	0.311*	0.464*	-0.464*	0.042	1.000			
Oil content %	0.383*	0.310*	-0.310*	-0.026	0.097	1.000		
Ash content %	0.116	0.055	-0.055	-0.116	0.042	-0.131	1.000	
Crude fiber content %	0.309*	0.353*	-0.353*	0.194	-0.238	-0.103	-0.258	1.000

*Correlation is significant at the 0.05 levelSignificant at 10% level of significance; ** Significant at 5% level of significance; *** Significant at 1% level of significance

Fig.1 Carbohydrate profile (amylose, amylopectin and total starch) and other chemical constituents of high RS containing maize genotypes. (A). Accumulation of amylose, amylopectin and total starch. (B). accumulation of protein, oil, ash and crude fiber content

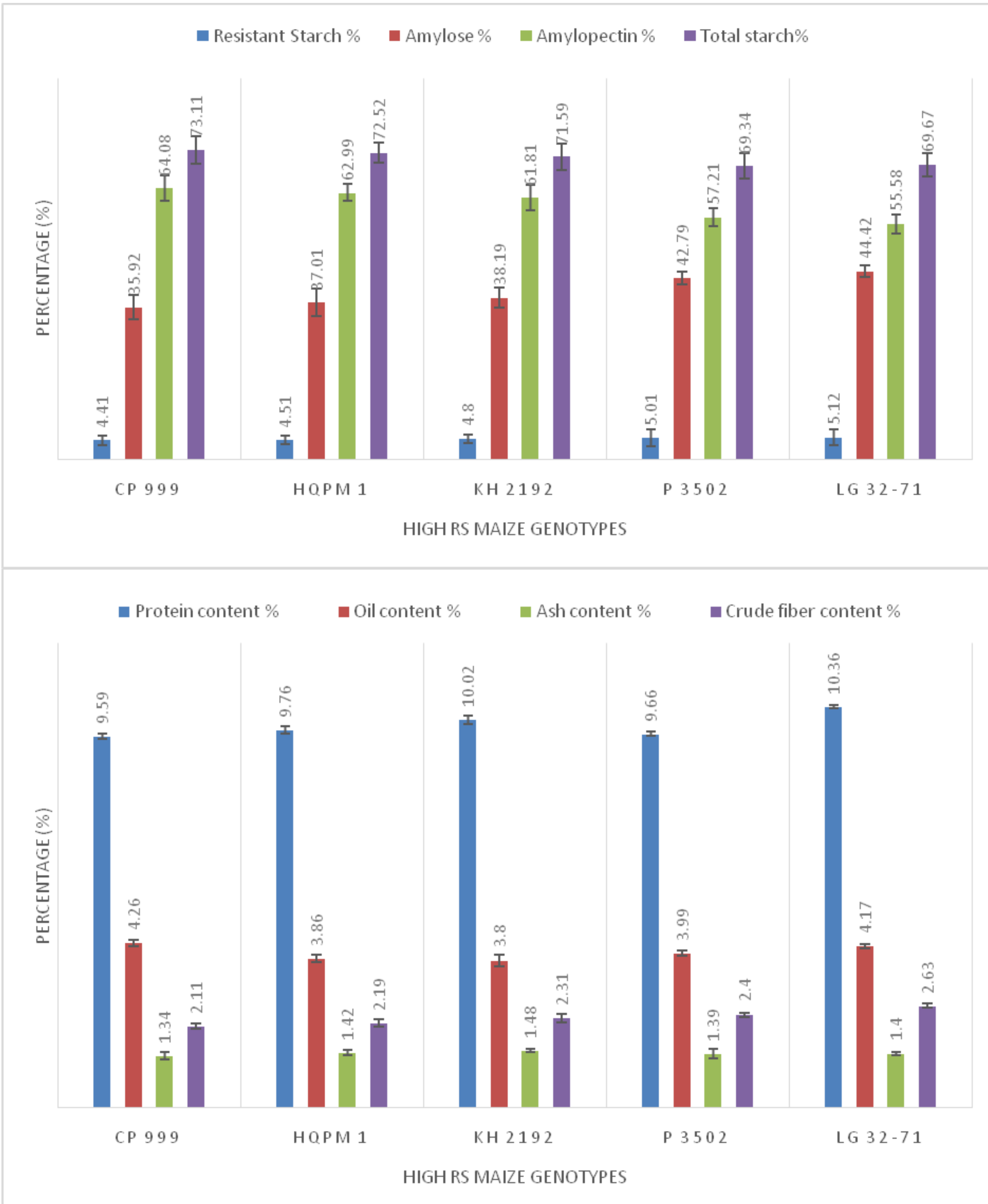
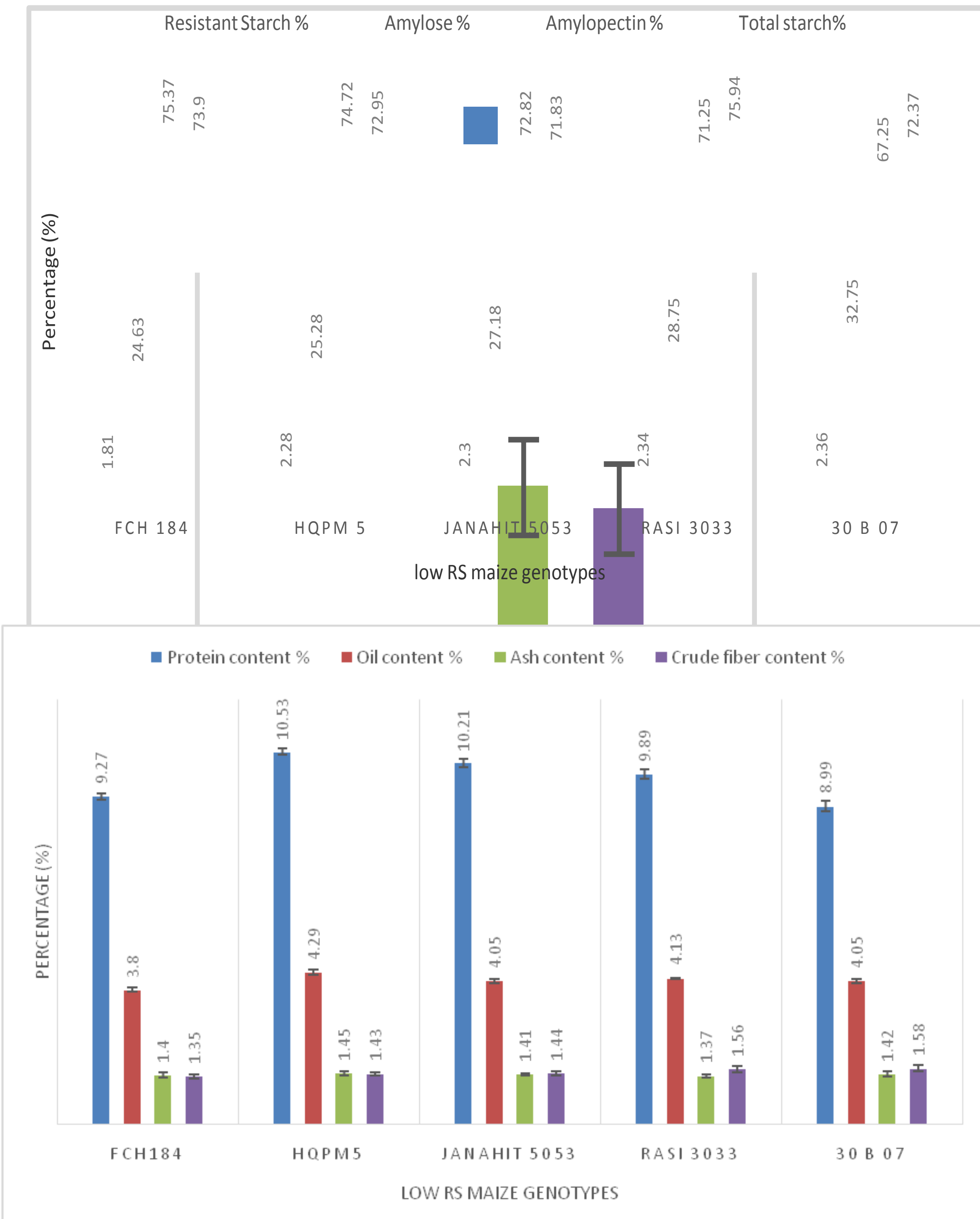


Fig.2 Carbohydrate profile (amylose, amylopectin and total starch) and other chemical constituents of low RS containing maize genotypes. (A). Accumulation of amylose, amylopectin and total starch. (B). accumulation of protein, oil, ash and crude fiber content



Protein is the second largest chemical component of the maize seed. This study visualized that protein content was significantly altered during the estimation, in both high and low RS containing maize genotypes. The protein content were fall in the range of 8.99 to 10.53% (figure 2 B) in the low RS containing maize genotypes, while its range recorded from 9.59 to 10.36% (figure 1 B) in high RS containing maize genotypes. Statistically, these data were recorded as significantly positive correlation with RS and amylose content and negatively correlated with amylopectin content in both high and low RS containing genotypes (Table 1 and 2). The increase of protein content in different maize genotypes were also reported by Saleem *et al.,* 2008; Idikut *et al.,* 2009; Berardo *et al.,* 2009; Ullah *et al.,* 2010.

Seed oil provides a concentrated source of energy for animals and during germination of seed, therefore there is interest in increasing the oil content of maize grain to increase the caloric content of the grain (Pollack *et al.,* 2005). Improving the quantity and quality of maize kernel oil content is consequently an important objective for the breeding programs. So that the estimation of oil content in seed become an important objective for improving the seed quality. We estimated the oil content in both high and low RS containing maize genotypes, observed oil content ranges 3.80 to 4.29% (figure 2 B) and 3.80 to 4.26%(figure 2 A) respectively. This was also showed positive correlation with RS and amylose content while negatively correlated with amylopectin content (table 1 and 2). Saleem *et al.,* 2008; KeShun, 2009; Berardo *et al.,* 2009; Ullah *et al.,* 2010; Ntuli *et al.,* 2013, also reported increase in oil content of different maize genotypes.

The present study also evaluated the accumulation of crude fiber content in both high and low RS containing maize genotypes. The present study showed that the high RS containing maize genotypes accumulated high crude fiber content (>2.0%) (Figure 1 B) than the low RS containing maize genotypes (<2.0%) (Figure 2 B). This result showed positive correlation with RS and amylose content while negatively correlated with amylopectin content (table 1 and 2). Ullah *et al.*, (2010) also reported the crude fiber variation in maize hybrids, ranged from 0.80 to 2.32%.The amount of ash content also play a significant role in maize seed quality. Ash contents levels were not significantly differ between the high and low RS containing maize genotypes and ranges from 1.32 to 1.48%(figure 1 B and 2 B). Similar results (0.70 – 2.50%)for ash content in different maize hybrids were reported by Egesel and Kahriman, 2012; Saleem *et al.,* 2008; KeShun, 2009; Berardo *et al.,* 2009; Ullah *et al.,* 2010; Ntuli *et al.,* 2013.

In conclusion, carbohydrate have a prominent component of maize seed in which high RS content genotypes show positive implications on human health and quality of seed. Present study suggested that high RS containing maize genotypes accumulated higher amylose content and lower accumulation of amylopectin content and vice-versa in the low RS containing maize genotypes. While the accumulation of starch content was unaffected by the accumulation of amylose and amylopectin content in both high and low RS containing maize genotypes. Protein, oil and crude fiber content showed positive correlation with RS and amylose content in both high and low RS containing maize genotypes. Although the ash content accumulation was not altered in both high and low RS containing maize genotypes.

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