Higher emergency vigor seed traits of sweet maize (*Zea mays* L) inbred lines in China by factor analysis and cluster analysis

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Abstract

Factor analysis and cluster analysis is broadly applied to define seed nutrient components that mostly determine the seed vigor in the field that can be used as breeding and selection criteria. By using factor analysis and cluster analysis, relationship of seed emergence percentage and seed traits of 122 sweet maize inbred lines were investigate in the experiment. Reserves compositions variability of the sweet maize kernels which processed by drying milling were determined. The results showed that the reserves compositions and emergency percentage were significantly different among the sweet maize inbred lines. The emergency percentage of sweet maize inbred lines was concerned with five principal components from factor analysis and the contribution rate of single kernel weight was the most important, which could account for 26.97%. Sweet maize inbred lines with emergency percentage higher than the 3rd quartile of the index could be classified into four groups by cluster analysis.

Keywords: sweet maize inbred lines, higher emergency vigor, principal components analysis, cluster analysis

Introduction

Sweet maize, also called sugar corn and pole corn (Singh et al, 2014), is a type of speciality cultivated maize, noted for its high sugar and nutrients content in the kernel, and thus generally feed people as one of the most important vegetable crops. It differs from field maize in many traits (Srdić et al, 2008). At the present, there is a growing trend in sweet maize production throughout China mainly due to its high economic return. China has already become the second producer of sweet maize in the world. In 2008, the estimated national sweet maize production amounted to 3.397 million metric tons. However, lower germination percentage and emergency percentage in the field has been a handicap in the sweet maize production in China.

Seed germination is both of a complex physiological process during which mobilization of nutrient and energy reserves (i.e., essential elements and macromolecular compounds) in the seed starts to carry out, and a crucial step for the successfully establishment of plant seedlings under different conditions. Besides degree of compatibility between two related traits obtained by calculating simple correlations, it is necessary to determine relations between independent variables and the traits observed as a dependent variable. Sweet maize is the genotype with naturally occurring recessive mutations (*sul*, *sul sel*, and *sh2*) in genes controlling conversion of sugar to starch inside the kernel endosperm (Feng et al, 2013). In sweet maize breeding process, application of relevant breeding criteria for higher seed vigor is of great importance to breeders and commercial growers. A large number of factors suggested to play an important role in germination of sweet maize include insufficient energy reserves, pericarp cracking, and membrane damage and reduced endosperm to embryo dry weight ratios due to both of reduced starch concentration and elevated sugar concentration (Azanza et al, 1996). The aim of the study is to analyze higher kernel traits for germination and emergence vigor improvement, nutrient and energy reserves concentration of 122 different sweet maize inbred lines and statistically classification based on the differences. The sweet maize varieties were obtained from seed savers exchange. Measurement was carried out in Fengyang county, Anhui province in China were compared on the basis of nutrients concentration and reserves by using factor analysis. Characterization of the kernel reserves variability will allow breeders to identify accessions with desirable characteristics of seed vigor of sweet maize inbred lines. Furthermore, characterization and grouping of the germplasms will allow breeders to avoid duplication in sampling populations and identify potential combining ability groups for final use suitability and provide a new pathway for evaluating the seed vigor of sweet maize inbred lines.

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Materials and Methods

Materials

122 sweet maize inbred lines were obtained at the Hainan winter breeding station (108°52'E;18°27'N) in Anhui Maize Breeding Engineering and Technology Research Center, Anhui Province, PR China. The inbred lines were inherited stably after five generations of self-pollination. Field experiments were conducted in 2014. Seed of the inbred lines were planted on 25 June 2014 at Agricultural Planting Station (117°40'E;32°86'N) in Anhui Science and Technology University. The experimental design was a randomized complete block with three replications. Three-row plots, 6.0 m long with 60 cm between rows, were overplanted with 72 seeds per row. Maize was milled with 100 mesh sieve and the obtained powder was stored at 4°C.

Composition analysis of maize seed

Seed weight was mesured by weight kernel by kernel. Total nitrogen concentration in seeds was determined according to the Kjeldahl method after digestion in sulfuric acid-H_2O_2, and crude protein concentration with a conversion factor of 6.25. Total phosphorus concentration in the digestion liquid samples was determined colorimetrically by the Mo-Sb-Vc-method, and total potassium concentration was determined using a flame photometer. Crude lipid concentration was measured by the Soxhlet extraction method.

Total starch concentration was analyzed by improved two-wavelength spectrophotometry method using potato starch (sigma; >99%) as standard. The results included mono- and disaccharides which were disregarded since they were only present in small quantities in raw cereal grains. Total soluble sugar concentration was measured based on the Anthrone method. All measurements were carried out in triplicate. Emergency percentage was determined by direct count of emerged seedlings of each genotype.

Statistical Analysis

The multivariate statistical technique called principal component analysis (PCA) is based on the calculation of linear combinations between the variables that explain the most variance of the data. As a result, data can be reduced to a set of new variables called principal components. The loadings of the principal component (PC) define the direction of the greatest variability. All compositions and properties were subjected to the principal component analysis to evaluate the relationships among them in order to identify the PC associated with the optimal separation of the grain components.

Cluster analysis was conducted to separate sweet maize varieties into several subgroups with respect to composition analysis and properties of kernel reserves to produce properties uniform varieties. Different clustering algorithms often produce substantially different clusters when the same data set is used.

The principal component analysis and cluster analysis were performed using SPSS 19.0 software package. All measurements were repeated three times, and the average values were used as data.

Results

Analysis on kernel weight, reserves and composition properties of sweet maize kernel

The composition analysis and properties of sweet maize kernels were listed in Table 1. The average, standard deviation (SD) and coefficient of variation (CV) of 122 sweet maize inbred lines were counted by the basic parameter estimation analysis. As shown in Table 1, greater difference in sugar reserve, emergency percentage of the seed kernel, starch reserve and sugar concentration were observed, and the CV of the variations were 32.90%, 63.64%, and 61.379%, respectively. The difference in seed weight was higher than that in water, nutrients, protein and fat concentration, and nutrients reserves, whereas, was lower than that in sugar, starch, fat and protein reserves, and sugar and starch concentration. This could lead to the difference in seed vigor of the 122 sweet inbred lines, and all of which would allow breeders to identify genotypes with desirable characteristics in breeding processes.

Factor Analysis

Results of factor analysis for different sweet maize inbred lines based on correlation matrix with 17 ker-

Table 1 - Property of kernel weight, eating quality, nutrient reserve and emergency percentage of sweet maize.

Index	Average	Range	Minimum	Maximum	Coefficient of variation (%)
Single kernel weight (g)	0.1519	0.3282	0.0635	0.3971	32.90
Water concentration (%)	6.88	5.76	3.56	9.32	16.75
Starch concentration (mg g^{-1})	365.2	663.3	19.00	682.3	50.39
Protein concentration (mg g^{-1})	194.4	153.7	129.6	283.3	15.76
Fat concentration (mg g^{-1})	109.4	156.2	40.80	197.0	27.59
Soluble sugar concentration (mg g^{-1})	147.5	397.2	32.00	429.2	61.37
Starch reserves (mg)	55.21	184.8	4.06	188.9	63.64
Protein reserves (mg)	29.78	85.66	11.00	96.65	41.28
Fat reserves (mg)	16.12	28.82	5.34	34.16	33.13
Sugar reserves (mg)	23.51	86.23	4.63	90.86	77.40
Nitrogen concentration (mg g^{-1})	31.10	24.59	20.74	45.33	15.76
Phosphorus concentration (mg g^{-1})	8.40	7.57	4.17	11.70	17.10
Potassium concentration (mg g^{-1})	5.65	6.34	1.82	8.17	26.28
Nitrogen reserves (mg g^{-1})	4.64	7.84	2.19	10.03	28.71
Phosphorus reserves (mg g^{-1})	1.26	2.34	0.57	2.91	30.50
Potassium reserves (mg g^{-1})	8.22	10.45	3.59	14.04	28.77
Emergency percentage (%)	31.8	94.4	0	94.4	71.12

Table 2 - Eigen values of the correlation matrix and its contribution and cumulative contribution.

Index	Initial eigen values	Percentage of variance	Cumulated percentage %
Factor 1	4.59	26.97	26.97
Factor 2	3.52	20.70	47.66
Factor 3	2.28	13.41	61.07
Factor 4	2.06	12.13	73.20
Factor 5	1.40	8.23	81.43

nel property indexes, were shown in Table 2. It can be observed that five factors, that is, five principal components, derived from the orthogonal transformation of the matrix, explained 81.43 % of the total variation according to their eigen values (factor 1: 27.0%; factor 2: 20.8%; factor 3: 13.8%, factor 4: 9.40%, and factor 5: 6.70%).

The coefficients are shown in Table 3. It could be appreciated the kernel composition analysis and emergency percentage associated to each principal component. Factor 1 significant positively related to starch, protein, fat, nitrogen, phosphorus and potassium reserves, was affected by kernel weight. Factor 2 significant positively related to fat, phosphorus and potassium concentration, and significant negatively related to soluble sugar concentration and sugar reserves, and the correlations were maximum significant. Factor 3 significant positively related to nitrogen and protein concentration. Factor 4 significant positively related to starch and water concentration, and starch reserves. And factor 5 positively related to emergency percentage, and obviously negatively related to phosphorus and potassium concentration.

Previous studies had only compared morphological properties and classification of sweet maize cultivars (Revilla and Tracy, 1995). While the factor analysis about the relationship between kernel properties and emergency percentage have not been reported. Our study ultimately obtained five principal components, in which the kernel weight is the most important principal component affecting the emergency percentage.

Since the factor analysis applied to kernel composition can indicate basic uncorrelated factors that determine the emergency percentage to the greatest extent, selection based on the results can efficiently predict the variable-related traits of sweet maize inbred lines. For example, if we divided each of the five factors into high and low levels, and in theory, all of the factors with one level could go with one another to form 32 combinations. Therefore, it would allow us identify inbred lines in the combinations with desirable characteristics such as higher soluble sugar concentration or higher vigor kernel properties related to emergency percentage.

Cluster analysis

In statistics, k-means clustering aims to partition n observations into k clusters in which each observation belongs to the cluster with the nearest mean, serving as a prototype of the cluster. 122 sweet maize inbred lines could be classified into 32 groups by K-means cluster analysis, and as a result, kernel nutrient characteristics of 22 inbred lines with emergency percentage higher than the 3rd quartile of the index selected from the total inbred lines, were shown in Table 4. Hierarchical cluster analysis is frequently used to build a hierarchy of naturally existing clusters. Figure 1 and Table 5 showed the presence of four clusters of the

Table 3 - Values of correlation coefficients of factorial matrix.

Index	Factor				
	1	2	3	4	5
Single kernel weight	0.848	-0.444	0.109	0.095	0.155
Water concentration	0.035	0.255	0.202	0.353	0.060
Starch concentration	-0.012	0.128	0.011	0.918	0.004
Protein concentration	0.077	-0.010	0.984	0.075	0.043
Fat concentration	0.078	0.813	-0.062	-0.137	0.193
Soluble sugar concentration	0.083	-0.726	-0.078	-0.408	-0.142
Starch reserves	0.414	-0.127	0.060	0.852	0.074
Protein reserves	0.744	-0.360	0.486	0.127	0.153
Fat reserves	0.825	0.328	0.030	-0.047	0.245
Sugar reserves	0.421	-0.779	-0.069	-0.279	-0.067
Nitrogen concentration	0.077	-0.010	0.984	0.075	0.043
Phosphorus concentration	0.094	0.657	-0.046	0.105	-0.493
Potassium concentration	-0.056	0.773	-0.137	-0.057	-0.512
Nitrogen reserves	0.876	-0.147	0.013	0.142	0.188
Phosphorus reserves	0.929	-0.054	0.085	0.146	-0.152
Potassium reserves	0.775	0.356	-0.073	0.001	-0.393
Emergency percentage	0.120	0.097	0.049	0.102	0.707

n=120, p=0.05, r=0.174; p=0.01, r =0.228.

Table 4 - Kernel nutrient characteristics of 22 sweet maize inbred lines with higher emergency percentage.

Index	Numbers and kernel nutrient characteristics of inbred lines with higher emergency percentage							
	4	3	1	1	2	7	2	2
Single kernel weight (g)	0.1302	0.1427	0.3044	0.3917	0.2355	0.1410	0.2035	0.1110
Water concentration (%)	6.49	7.16	8.12	9.00	8.08	7.04	7.64	8.13
Starch concentration (mg g^{-1})	508.4	389.7	620.5	201.0	42.7	354.0	672.6	128.5
Protein concentration (mg g^{-1})	204.8	195.7	197.2	246.7	208.5	171.2	202.1	247.4
Fat concentration (mg g^{-1})	123.6	121.8	61.3	87.2	61.7	113.3	60.5	136.6
Soluble sugar concentration (mg g^{-1})	109.7	248.2	205.9	99.3	141.7	104.6	130.7	141.5
Starch reserves (mg)	64.09	55.85	188.86	78.74	10.58	49.65	136.70	13.72
Protein reserves (mg)	26.70	27.85	60.02	96.65	49.24	24.14	41.19	27.41
Fat reserves (mg)	16.42	17.72	18.67	34.16	14.07	15.80	12.32	14.81
Sugar reserves (mg)	139.9	348.7	626.6	389.2	336.3	145.9	265.4	153.2
Nitrogen concentration (mg g^{-1})	32.76	31.31	31.55	39.48	33.35	27.38	32.33	39.58
Phosphorus concentration (mg g^{-1})	8.53	9.59	5.59	7.42	6.30	8.72	7.07	9.28
Potassium concentration (mg g^{-1})	5.01	7.08	2.16	2.86	3.17	5.76	3.08	6.33
Nitrogen reserves (mg g^{-1})	4.22	4.56	7.23	10.03	5.82	4.73	5.43	4.01
Phosphorus reserves (mg g^{-1})	1.11	1.39	1.70	2.91	1.48	1.24	1.44	1.01
Potassium reserves (mg g^{-1})	6.68	10.28	6.56	11.21	7.29	8.12	6.38	6.84
Emergency percentage (%)	72.92	68.98	58.33	55.56	63.89	63.69	80.56	76.39

22 sweet maize inbred lines with higher emergency percentage by Ward's minimum variance method. The first cluster with the least distance was formed by 14 inbred lines. The second cluster was formed by 4 inbred lines. The third one was formed by 3 inbred lines. The last cluster grouped only 1 inbred line.

Discussion

Unlike field maize cultivars, sweet maize is picked at the milk stage to keep the special delicious and fragrant, and used as a fresh or canned vegetable, rather than a grain (Feng et al, 2013; Singh et al, 2014). Since maturation process of sweet maize genotypes with sul involves biochemical conversion of sugar into starch, shelf-life of sweet maize is very short and must be harvested before the kernels become tough and starchy (Singh et al, 2014). However, sweet maize genotypes either with *sul sel* or *sh2* have also been shown to retain higher sugar and moisture concentrations at typical harvest maturity and for longer postharvest periods, and then further reduce seed vigor under the field conditions, include field emergency, seedling vigor, stand uniformity and seedling growth rate (Azanza et al, 1996).

Yield benefits from uniformly plant emergence in maize (*Zea mays* L) have received considerable attention (Liu et al, 2004). Liu et al (2004) have found that, total yield was reduced 8% when one of six maize plants was delayed in emergence by four leaves. Since germination and seedling stage represent the most vulnerable periods in a plant's life cycle, any life-history trait that influences either emergency or germination percentage will therefore affect successful establishment of maize progenies (Vange et al, 2004). Seed size and composition of seed reserves are expected to vary among wild species in relation to their biological fitness for germination (Vange et al, 2004; Münzbergová et al, 2010; Soriano et al, 2011), seedling traits (Vange et al, 2004; Naegle et al, 2005; Münzbergová et al, 2010; Soriano et al, 2011) and grain yield of cereals (Zhang et al, 1990).

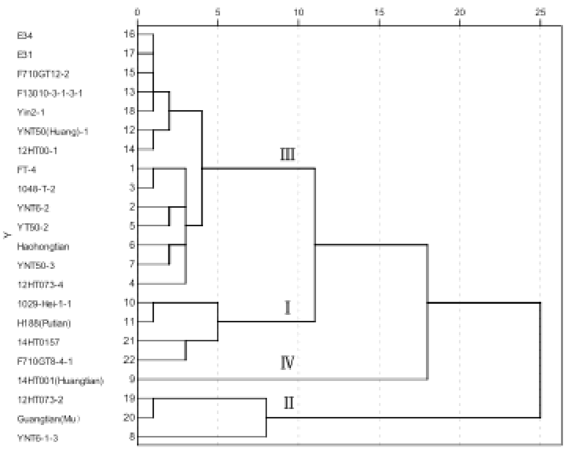

Figure 1 - Cluster Analysis of the 22 sweet maize inbred lines with higher emergency percentage.

By the identification of kernel weight and nutrient characteristics that affect emergency percentage of sweet maize to the greatest extent, their importance, as breeding criteria in the process and selection of superior genotypes, is emphasized. In the experiment, 122 sweet maize inbred lines behaved differently in terms of reserves concentration and some of their physicochemical properties of the kernel. Principal component analysis identified five components that explained 81.43% of the total variation among sweet maize kernel traits. The results suggested that the application of multivariate statistics in sweet maize variety characteristics was practical and effective. However, the cluster analysis about the emergency percentage of sweet maize inbred lines in China also has not been reported. Sweet maize can lose 50% of its sugar concentration in 24h in room temperature (Singh et al, 2014). As was previously observed, field emergency was negatively correlated with sugar concentration, while positively correlated with starch concentration at maturity stage (Azanza et al, 1996). Since total starch reserves account for 75% of the kernel weight, as a consequence, kernel weight was also positively correlated with field emergency (Azanza et al, 1996). This agrees with the results in this experiment. Simultaneous selection for eating quality and field emergency is needed in order to make progress in both of the two properties. Furthermore, various factors can affect the relationship among kernel characteristics and emergency percentage, including different endosperm mutations in the selection, environmental factors variations, sufficient kernel fill (Azanza et al, 1996) and the applied statistical analysis used to reveal the existing links among the observed > traits. Mobilization of nutrient reserves may be associated with the changes in seed vigor (Walters et al, 2005) and *vice versa*. Therefore, it is most desirable to perform selection at dynamic germination stage and under the conditions where sweet maize inbred lines will grow in China.

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