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Relationships between changes of kernel nutritive components and seed vigor during development stages of F_1 seeds of *sh*$_2$ sweet corn*

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Abstract: The changes of kernel nutritive components and seed vigor in F_1 seeds of *sh*$_2$ sweet corn during seed development stage were investigated and the relationships between them were analyzed by time series regression (TSR) analysis. The results show that total soluble sugar and reducing sugar contents gradually declined, while starch and soluble protein contents increased throughout the seed development stages. Germination percentage, energy of germination, germination index and vigor index gradually increased along with seed development and reached the highest levels at 38 d after pollination (DAP). The TSR showed that, during 14 to 42 DAP, total soluble sugar content was independent of the vigor parameters determined in present experiment, while the reducing sugar content had a significant effect on seed vigor. TSR equations between seed reducing sugar and seed vigor were also developed. There were negative correlations between the seed reducing sugar content and the germination percentage, energy of germination, germination index and vigor index, respectively. It is suggested that the seed germination, energy of germination, germination index and vigor index could be predicted by the content of reducing sugar in sweet corn seeds during seed development stages.

Key words: *Sh*$_2$ sweet corn, Kernel nutritive component, Seed vigor, Time series regression (TSR) analysis
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INTRODUCTION

High vigor seeds have distinct growth ascendancy and production potential, which can improve field emergence, resist environmental stress, and increase crop yields (Gupta *et al*., 2005; Rodo and Marcos, 2003). Sweet corn with *sh*$_2$ gene is extensively planted in China and all over the world owing to their favorite tasting. However, low starch content in endosperm of sweet corn caused low seed vigor, which induced low germination rate and poor emergence (Parera and Cantliffe, 1994). Seed vigor could be improved by seed treatment such as soaking, priming, coating and infusing with the organic solvents (Hartz and Caprile, 1995; Fan *et al*., 1998; Zhang C.F. *et al*., 2007). The research on seed vigor of sweet corn was frequently focused on pre-sowing treatments. However, seed vigor is affected during seed development. When seeds are fully mature, their vigor usually reaches the highest level. Most of the studies on kernel development were conducted to study kernel quality and sweet corn yield for providing theoretical basis for quality breeding, food processing and suitable harvest time (Wu and Chen, 1999; George *et al*., 2003). Little information is available on relationships between the changes of kernel nutritive components and seed vigor during seed development and maturation in *sh*$_2$ sweet corn. Time series regression (TSR) analysis method revealed relationships among main factors quantificationally by analyzing the data with time series, and its major merit is simplifying complex problems into dependence relationship among several principal factors (Karnezos

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and Matches, 1992; Liu, 1997). Analysis of relationships between the changes of kernel nutritive components and seed vigor during seed development in sh_2 sweet corn by TSR has not yet been reported as far as we know.

In present study, the changes of kernel nutritive components and seed vigor during seed development period in F_1 seeds of sh_2 sweet corn were determined, and their TSR equations were established for investigating the effects of kernel nutritive components on seed vigor. Predicting vigor expression at different development stages will provide theoretical basis for the production of sweet corn seeds with high vigor.

MATERIALS AND METHODS

Materials

Supersweet corn seeds (F_1 hybrid with sh_2 gene) from cv. Supersweet 2018 and Shuxuan 1 were used. Their respective parental inbreds were planted in the field with similar soil conditions and usual management. The planting was performed with a density of 4500 plants per 666.7 m^2 at Hangzhou Vegetable Science Institute in Zhejiang Province, China. Seeds of two F_1 hybrids were produced after synchronous hand cross-pollination.

Seed harvest

Corn ears were harvested at various development stages between 14 to 42 d (at an interval of 4 d) after pollination (DAP). At each harvest, 10 plants were randomly chosen and the first ear from the top of the plant was harvested by hand. Seeds from the middle part of corn ears were threshed by hand, then air-dried, and mixed for further tests.

Germination tests

Germination tests were conducted with three replicates, each consisting of 50 seeds placed in a 12 cm×18 cm×9 cm germination box containing wet sand. After that, seeds were incubated in a germination chamber at 25 °C for 7 d. The number of germinated seeds was counted daily, and then energy of germination and germination percentage were calculated after 4 and 7 d, respectively. Based on the number of germinated seeds, germination index ($GI=\sum(G_t/T_t)$, where G_t is the number of the germinated seeds on Day t, and T_t is time corresponding to G_t in days) and vigor index ($VI=GI\times H_s$, where H_s is shoot height) were calculated (Zhang S. *et al*., 2007; Muharrem *et al*., 2008). Energy of germination was the percentage of the number of germinating seeds 4 d after planting relative to the number of seeds tested.

Kernel nutritive component measurement

Evaluation of kernel nutritive components was performed on 3 replications of 20 seeds at each harvest. Soluble protein was extracted from the seeds and determined according to the method of Coomassie brilliant blue G-250 staining (Li, 2000). Contents of total soluble sugar, reducing sugar and starch were determined by 3,5-dinitrosalicylic acid method (Jiang, 1999).

Statistical analysis

A regression analysis for the relationships between kernel nutritive components and seed vigor was conducted by the method of TSR in SAS (version 8.0).

RESULTS

Changes of nutritive components during seed development

Both of total soluble sugar and reducing sugar contents in the kernels decreased gradually along with the seed development in Supersweet 2018 and Shuxuan 1, but appeared unchanged or slightly changed during 26 to 42 DAP (Fig.1a).

Starch, transformed from soluble sugar during seed development, accumulated in seed endosperm. During seed development, starch content in the kernels gradually increased with small changes from 14 to 42 DAP. Similarly, the soluble protein content of the kernels harvested in different development stages changed slightly from 7.26% to 7.92% in Supersweet 2018 and from 7.59% to 8.85% in Shuxuan 1 (Fig.1a).

Changes of germination and vigor during seed development

Along with seed development, germination energy, germination percentage, germination index and vigor index had a gradual increase trend; two cultivars had similar changes except at 22 DAP (Fig.1b). All the four parameters had low levels at 14 DAP in both

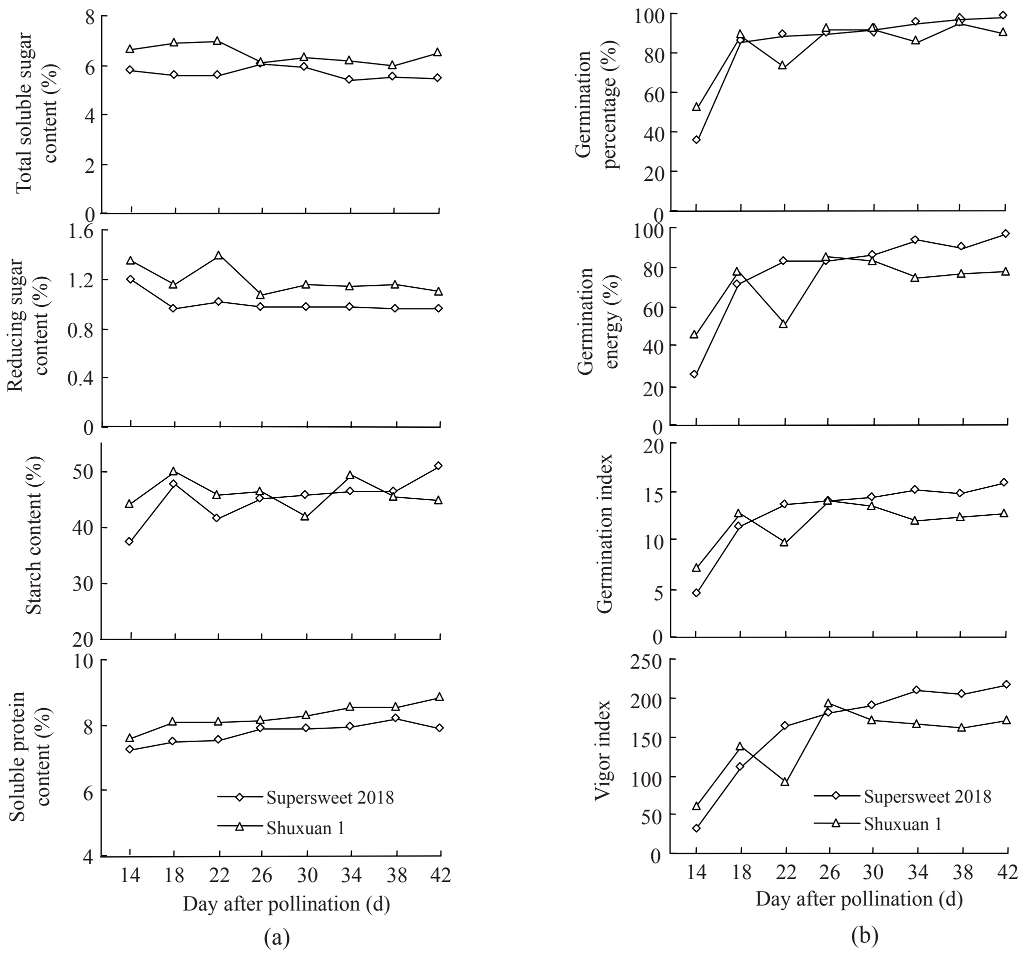

Fig.1 The changes of (a) the nutritive components (total soluble sugar, reducing sugar, starch, and soluble protein) and (b) germination and vigor (germination percentage, energy of germination, germination index, and vigor index) in kernels of Supersweet 2018 and Shuxuan 1 during seed development

cultivars, and germination percentage was only 34.7% in Supersweet 2018 and 51.3% in Shuxuan 1, respectively. Rapid increases in germination energy, germination percentage, germination index and vigor index were observed between 14 and 18 DAP. All the four parameters reached higher level at 26 DAP, and then changed slightly after 26 DAP.

TSR analysis between kernel nutritive components and seed vigor

The relationships between kernel nutritive components and seed vigor from 14 to 42 DAP in Supersweet 2018 and Shuxuan 1 were analyzed using TSR method (Table 1). Among all 8 equations, X_2 (reducing sugar) in 6 equations, X_3 (starch) in 3 equations and X_4 (soluble protein) in 2 equations had a significant contribution to the corresponding equations (P<0.05), respectively, while X_1 (total soluble sugar) failed to contribute to all the equations (Table 1). The contribution of X_2 (reducing sugar) to the significant equations was larger than that of the other three variables, reaching 75%. The equations with significant X_2 had values of R^2 ranging from 0.6237 to 0.9367, suggesting that the equations had a higher accordant level with data, and reducing sugar among nutritive components was the main factor affecting seed vigor. According to the above results, simpler equations were developed with TSR by eliminating factors with slight effects on seed vigor (Table 2). Reducing sugar had significant contributions (P<0.05) to all 8 equations. When compared with equations in Table 1, R^2 values in Table 2 slightly changed; however, equations were easier to be used. Seed vigor can be predicted according to the data of reducing sugar obtained during seed development stages.

Table 1 The time series regression equations between kernel nutritive components and seed vigor during development of Supersweet 2018 and Shuxuan 1

Variety	Parameters	Time series regression equations	R^2
Supersweet 2018	Germination percentage	$\hat{Y}_1=-11.9095-12.8533X_1-87.2261X_2+1.6293X_3+21.0856X_4$ (b_0=0.8976, b_1=0.1558, b_2=0.0014, b_3=0.0171, b_4=0.0453)	0.9367
	Energy of germination	$\hat{Y}_2=-75.1143-19.8869X_1-43.6137X_2+2.1360X_3+23.7112X_4$ (b_0=0.5867, b_1=0.1264, b_2=0.2070, b_3=0.0249, b_4=0.1176)	0.8897
	Germination index	$\hat{Y}_3=-4.5712-2.1622X_1-12.0763X_2+0.2357X_3+3.6027X_4$ (b_0=0.8313, b_1=0.2674, b_2=0.0293, b_3=0.0955, b_4=0.1316)	0.9005
	Vigor index	$\hat{Y}_4=-494.6744-21.7958X_1-112.6757X_2+3.4844X_3+90.7512X_4$ (b_0=0.2667, b_1=0.5623, b_2=0.2860, b_3=0.2015, b_4=0.0625)	0.8730
Shuxuan 1	Germination percentage	$\hat{Y}_1=76.7246+3.0901X_1-78.2951X_2+0.0854X_3+9.7703X_4$ (b_0=0.4747, b_1=0.6862, b_2=0.0216, b_3=0.9336, b_4=0.2593)	0.6237
	Energy of germination	$\hat{Y}_2=213.4506+4.1800X_1-122.2558X_2-0.3551X_3+0.0295X_4$ (b_0=0.0123, b_1=0.4783, b_2<0.0001, b_3=0.6364, b_4=0.9961)	0.7880
	Germination index	$\hat{Y}_3=28.9537+0.7522X_1-16.7996X_2-0.0227X_3+0.0308X_4$ (b_0=0.0795, b_1=0.4820, b_2=0.0019, b_3=0.8820, b_4=0.9809)	0.6895
	Vigor index	$\hat{Y}_4=282.6150+5.8546X_1-290.8102X_2-0.8196X_3+26.2725X_4$ (b_0=0.2948, b_1=0.7538, b_2=0.0016, b_3=0.7445, b_4=0.2181)	0.7696

X_1, X_2, X_3 and X_4 were total soluble sugar, reducing sugar, starch and soluble protein contents, respectively; $\hat{Y}_1$, $\hat{Y}_2$, $\hat{Y}_3$ and $\hat{Y}_4$ were germination percentage, energy of germination, germination index and vigor index, respectively; b_0, b_1, b_2, b_3 and b_4 were P values for intercept, X_1, X_2, X_3 and X_4, respectively

Table 2 The time series regression equations between seed reducing sugar content and seed vigor during development of Supersweet 2018 and Shuxuan 1

Variety	Parameters	Time series regression equations	R^2
Supersweet 2018	Germination percentage	$\hat{Y}_1=231.5300-147.3489X_2$ (b_0<0.0001, b_2<0.0001)	0.8796
	Energy of germination	$\hat{Y}_2=185.7707-108.4236X_2$ (b_0<0.0001, b_2=0.0012)	0.8143
	Germination index	$\hat{Y}_3=31.4047-18.7533X_2$ (b_0<0.0001, b_2=0.0001)	0.8633
	Vigor index	$\hat{Y}_4=352.9968-198.9216X_2$ (b_0=0.0004, b_2=0.0114)	0.8480
Shuxuan 1	Germination percentage	$\hat{Y}_1=188.3693-88.3884X_2$ (b_0<0.0001, b_2=0.0008)	0.5906
	Energy of germination	$\hat{Y}_2=207.5386-113.8824X_2$ (b_0<0.0001, b_2<0.0001)	0.7804
	Germination index	$\hat{Y}_3=30.3665-15.6029X_2$ (b_0<0.0001, b_2<0.0001)	0.6799
	Vigor index	$\hat{Y}_4=516.5426-312.7926X_2$ (b_0<0.0001, b_2<0.0001)	0.7447

X_2 was reducing sugar content; $\hat{Y}_1$, $\hat{Y}_2$, $\hat{Y}_3$ and $\hat{Y}_4$ were germination percentage, energy of germination, germination index and vigor index, respectively; b_0 and b_2 were P values for intercept and X_2, respectively

DISCUSSION

Seed development starts from zygote. During the development of seeds, a large number of nutrients gradually accumulate inside of the seeds, and morphological and physiological changes in seeds take place. Finally, seeds gradually reach maturation and have high vigor (Huang and Fu, 1992). Generally, corn seeds reach to complete maturity stage at about 40 DAP; therefore, the changes of nutritive components and vigor of sweet corn seeds from 14 to 42 DAP were estimated in present study.

In different stages of seed development, the contents of nutrient components in seeds changed, and there were similar change trends in nutritive components between the two sh_2 hybrids, Supersweet 2018 and Shuxuan 1. The present study shows that the contents of total soluble sugar and reducing sugar decreased gradually throughout the development stages, and the contents of starch and soluble protein in seeds increased, which is consistent with previous reports (Yan, 2001).

Realizing the changes of seed vigor and germination percentage during seed development stages can estimate whether seeds reach full maturation, which plays an important role in seed production. The present study shows that seeds of both Supersweet 2018 and Shuxuan 1 could be harvested at 18 DAP, if an 85%-germination was selected as the criteria for harvest. However, low germination index and vigor index were observed in both genotypes at this stage. Therefore, 18 DAP was not a suitable time for harvesting. For evaluating seed quality of development stages, several parameters of seed vigor should be simultaneously considered. The differences of germination percentage, energy of germination, germination index and vigor index were not obvious in supersweet corn seeds harvested between 26 and 42 DAP. However, the seeds harvested at 38 DAP tended to have their greatest vigor, suggesting 38 DAP to be the optimal time for harvesting sh_2 corn seeds. Regression equations between seed reducing sugar content and seed vigor were obtained in the present study; however, all coefficients of reducing sugar in different parameters of vigor were negative. It suggests that the seed germination, energy of germination, germination index and vigor index could be predicted by the content of reducing sugar in sweet corn seeds, and that the lower the content of reducing sugar is, the higher the seed vigor is, during seed development stages.

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