Comparison of starch granule sizes of maize seeds between parental lines and derived hybrids suggests a maternal inheritance trend

Running head: Starch granule sizes in maize parental and hybrid seeds

Liangjie Niu, Wei Wang*

National Key Laboratory of Wheat and Maize Crop Science, College of Life Sciences, Henan Agricultural University, Zhengzhou 450002, China

Correspondence

Prof. Wei Wang; email: wangwei@henau.edu.cn

ABSTRACT

Maize (*Zea mays*) starch is an important agricultural commodity that serves as food, feed, and a raw material for industrial purposes. It is organized into starch granules (SG) inside amyloplasts and is highly accumulated in endosperms. Maize hybrids, which exhibits heterosis over their parents, are globally grown due to higher vigor of the F1 plants. However, the parental effect on the size of SG in F1 hybrid seeds remains unclear. Here we compared the seed SG sizes among two parental inbred lines (Chang7-2 and Zheng58) as well as their reciprocal hybrids. SG was observed *in situ* and *in vitro* with SEM. The size of seed SG in hybrids was more like that of female parents, especially for large SG population. Thus, the control of SG size exhibits a maternal inheritance trend in the context of plastid (amyloplast) inheritance. Our results provide some insight on selecting parental inbred lines for breeding maize hybrids with different SG sizes.

Keywords: Maize seeds, hybridization, starch granule size, maternal effect, microscopy

Abbreviations: SG, starch granules.

23 # INTRODUCTION

24 Starch is the predominant carbohydrate reserve in plants (Zeeman et al., 2010). It consists of
25 glucose residues linked to each other by α-1,4-linkages (amylose) with occasional α-1,6-
26 branches (amylopectin) (Zeeman et al., 2010). In addition to as food and feed, starch is used as
27 texturizer, gelling agent, thickener, adhesive, moisture-retainer, biofuels etc. (Niu et al., 2019).

28 Starch accumulates in amyloplasts in a semi-crystalline granular form, which is divided into
29 two forms: storage starch and transient starch (Zeeman et al., 2010). Storage starch granule (SG)
30 mainly exists in cereal seeds, storage roots, and tubers, with a highly varied size of 2 to 50 μm
31 dependent on plant species (Seung and Smith, 2019). Transient SG is accumulated in leaf
32 chloroplast and is usually small (~2 μm) with a uniformly flattened disc shape among plant
33 species (Seung et al., 2017; Yu et al., 2017; Zeeman et al., 2002). The SG with different sizes
34 can be used for specific applications. Large SG with more amylose, higher peak, trough and
35 final pasting viscosities, and lower gelatinization temperature than small SG (Cai et al., 2014),
36 resulting in different bread- and noodle-making quality (Zi et al., 2019). Fine SG can be used
37 for generating thin films (Lindeboom et al. 2004) and fatty mouthfeel (Ma et al., 2006).

38 Many studies have demonstrated that the morphology and size of storage SG are mainly
39 determined by species origin (Zeeman et al., 2010), biochemistry and physiology of plants
40 (Gregorová et al., 2006; Alcázar-Alay and Meireles., 2015; Zhao et al., 2018). Some QTLs and
41 protein (e.g. SUBSTANDARD STARCH GRAIN6, starch synthase I) controls SG size have
42 been identified in maize kernels, rice and wheat endosperm using mutant (Liu N et al. 2018;
43 Matsushima R et al. 2016;McMaugh SJ et al. 2014). However, little reports about the effect of
44 parental inbred lines on the seed SG size in derived hybrids in maize.

45 Maize (*Zea mays*) is globally grown for its high seed starch content. Its hybrids exhibit
46 heterosis over parental lines in plant growth and development, yield and stress tolerance
47 (Hoecker et al., 2006). The hybrid kernel traits, especially weight and size, are mostly affected

by female parents (Zhang et al., 2016). Here two inbred lines Chang7-2 (C7-2) and Zheng58 (Z58) and their derived hybrids were used to instigate the parental effect on the SG sizes by scanning electron microscopy.

MATERIALS AND METHODS

Maize inbred lines C7-2 and Z58 were grown and reciprocally crossed as described previously (Ning et al., 2017). Mature seeds of C7-2, Z58, C7-2 × Z58, and Z58 ×C7-2 were used here. Scanning electron microscopy (SEM) observation of seed SG *in situ* and *in vitro* was exactly performed as our recent work (Niu et al. 2019). The SG size was expressed as spherical diameter by measuring at least 300 granules. All assays were conducted in at least three biological replicates. Statistically significant differences were accessed by the Student's t-test ($p<0.05$).

RESULTS

The seeds of C7-2 and Z58 significantly differ in shape, size and weight. The C7-2 seeds were like triangle and longer, whereas the Z58 seeds were rounder and wider (Fig. 1a-1c). The derived hybrids were like the female parent in morphology but with an increased size and weight (Fig. 1d-1e). Moreover, the proportion of the endosperm in the hybrid seeds was determined by the female parent (Fig. 1f).

The seed SG *in situ* of the four materials were in spherical shape (Fig. 2a), but the SG surface of the parental seeds was smooth but was rough in the hybrids with more membrane debris. The size of SG in all samples ranged from 4.5 to 19.5 μm, and the hybrid seeds were more similar to the female parent in the average SG size and distribution (Fig. 2b- 2c), especially the ratio of large SG population (Fig. 3d), exhibiting a maternal inheritance trend.

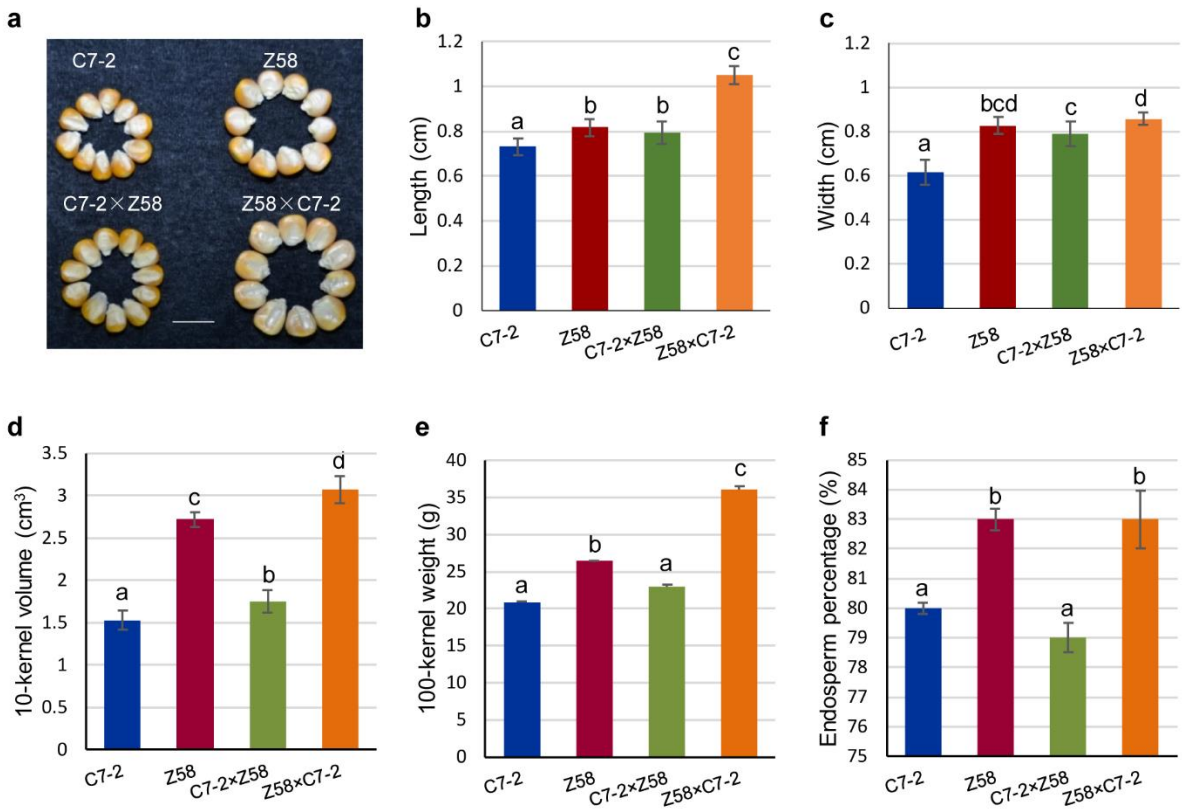

Figure 1. Phenotypic analysis of seeds. **a** Seeds of two inbred lines and their reciprocal hybrids. Bar = 1 cm. **b** The length of kernels (mean ±SD, n=50). **c** The width of kernels (mean ±SD, n=50). **d** The 10-kernel volume (mean ±SD, n=5). **e** The 100-grain weight (mean ±SD, n=5). **f** The ratio of endosperm in seed (mean ±SD, n=5).

The seed SG *in vitro* in all samples were mostly polygonal with similar surface features, because the membrane debris on the surface of the SG were removed during the isolation (Fig. 3a). The size of the isolated SG varied from 4.7 to 27.98 μm (Fig. 3b), greater than that *in situ*. Even so, the size distribution of the SG *in vitro* was comparable to the *in vivo* results (Fig. 3d). Additionally, the SG size correlated with the 100-kernel weight and the endosperm sizes, with correlation coefficients of 0.5595 ($p<0.05$) and 0.5773 ($p<0.05$), respectively.

DISCUSSION

Maize seeds consist of up to 78% starch that is overwhelmingly accumulated in the endosperm. The endosperm plays a key role in determining seed size (Berger et al., 2006). Large

SG is the major determinant of wheat endosperm weight (Chojkecki et al., 1986). Female parent significantly influences maize hybrid seed traits through altering endosperm cell number (Jones et al., 1996).

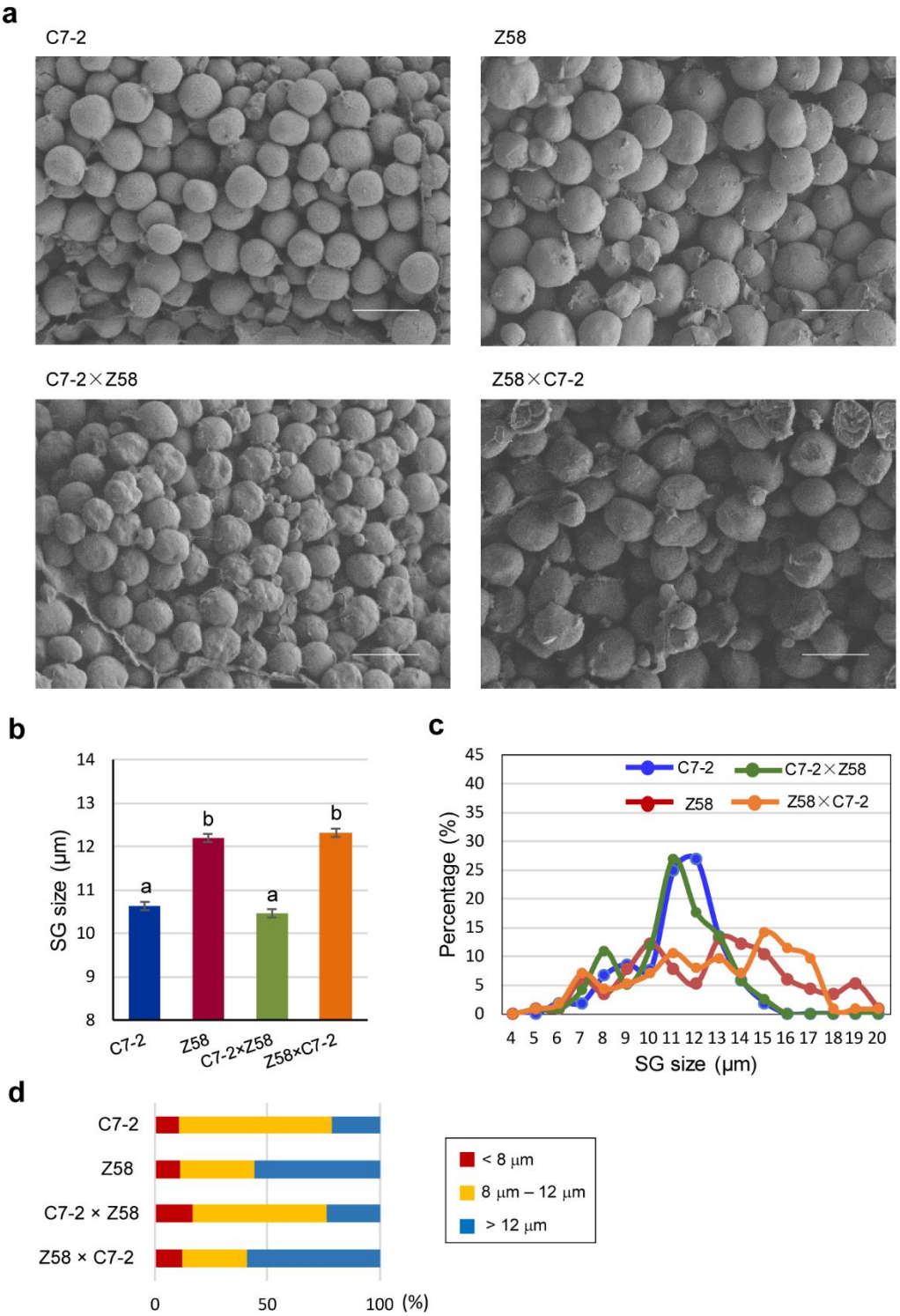

Figure 2. The morphology and size of seed SG *in situ*. **a** SEM observation. Bar = 20 μm. **b** Average SG diameter of. **c** SG size distribution. **d** The size change in different SG populations.

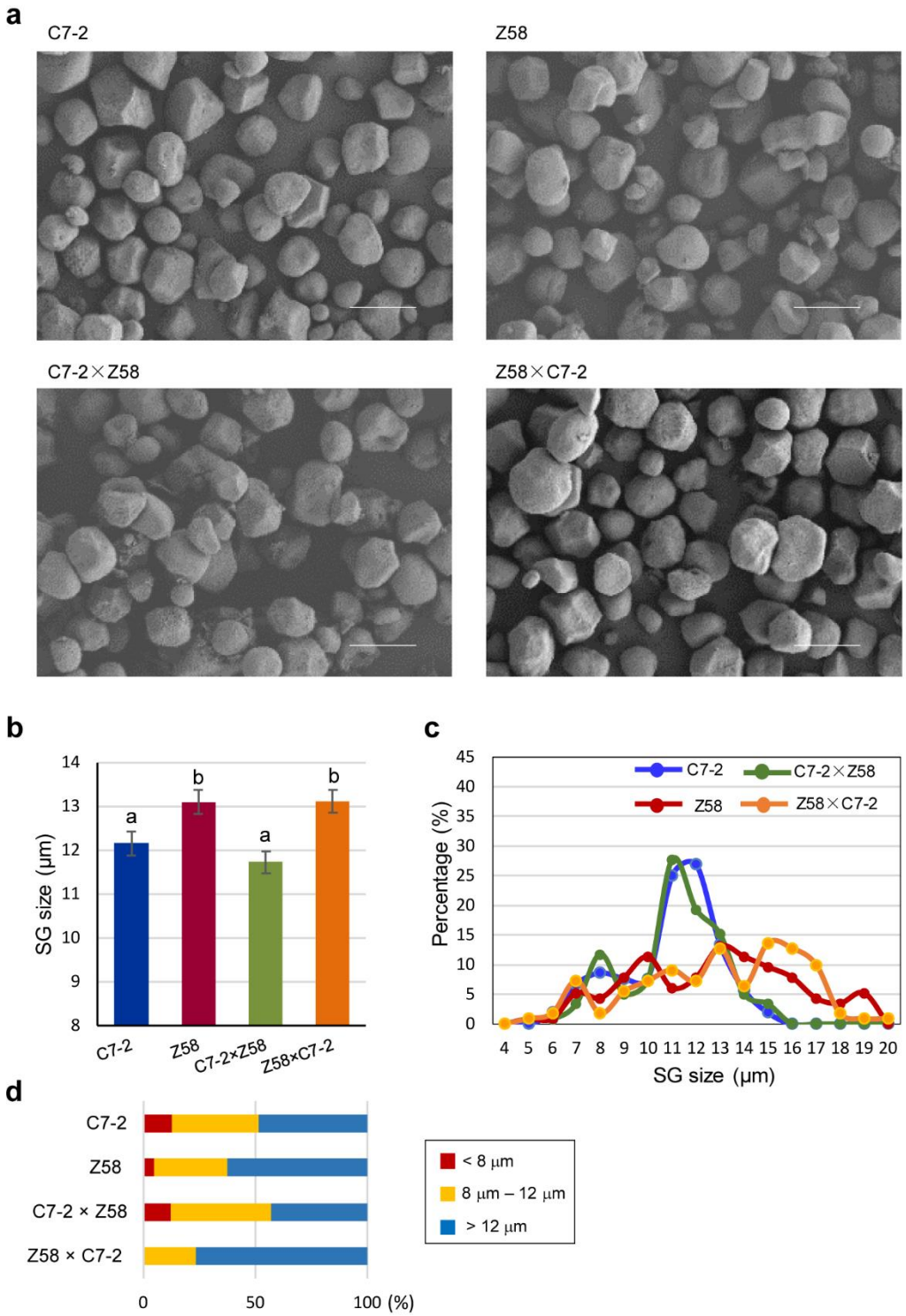

Figure 3. The morphology and size of seed SG in vitro. **a** SEM observation. Bar = 20 μm. **b** Average SG diameter of. **c** SG size distribution. **d** The size change in different SG.

DISCUSSION

Maize seeds consist of up to 78% starch that is overwhelmingly accumulated in the endosperm. The endosperm plays a key role in determining seed size (Berger et al., 2006). Large SG is the major determinant of wheat endosperm weight (Chojkecki et al., 1986). Female parent significantly influences maize hybrid seed traits through altering endosperm cell number (Jones et al., 1996).

In the present study we found the SG size in hybrid seeds was more affect by the female parent. Two reasons may explain this phenomenon. First, the endosperm is a triploid tissue that receives two genomes from mother and one genome from the paternal side (Li and Li, 2015). Gómez et al. (2002) found endosperm transfer cell can facilitate the transport of maternal solutes and nutrients at the interface between maternal tissues and the endosperm. Second, starch is synthesized and stored in plastids (chloroplasts/amyloplasts) in leaves or seeds (Seung and Smith, 2019). Plastids are maternally inherited in plants, as observed in Epilobium plants by electron microscopy (Schmitz and Kowallik 1987). It is suggested that maternal plastid inheritance is the elimination of paternal plastids during fertilization or the exclusion of plastids from germ cells during pollen development (Kuroiwa 2010). However, the mechanisms of parental inbred lines on the SG size in their hybrid seeds remains unclear in the case of maize.

The size and shape of the SG are controlled by multiple factors, such as botanical species origin, biochemistry and physiology of plants, and SG properties (Ellis et al., 1998; Nordmark and Ziegler., 2002; Ji et al., 2003a, 2003b; Lu et al., 2011; Cai et al., 2014). Super-sweet, pop, waxy, and dent maize differ significantly in size and shape of seed SG (Cui et al., 2014). The SG size and morphology in maize seeds are affected by nitrogen levels (Zhao et al., 2018). A significant SNPs within genes GRMZM2G419655 and GRMZM2G511067 was identified as functional markers in screening maize SG size (Liu et al., 2018). In rice, amyloplast membrane protein SUBSTANDARD STARCH GRAIN6 can control SG size in endosperm (Matsushima et al., 2016). In wheat, starch synthase I can change the morphology, size and fine structure of

starch in wheat endosperm by affecting the amylose content (McMaugh et al., 2014). However, the mechanism controlling the size and shape is largely unclear.

It is needed to note that maize seed SG were generally spherical *in situ* with and polygonal *in vitro* with comparative sizes as observed previously (Li et al. 2007; Zhang et al., 2011; Paraginski et al., 2014; Zhao et al., 2018; Niu et al., 2019). The large SG took a slight greater proportion in the isolated SG, possibly because some of the fine SG was lost during the isolation and all SG may somewhat swell from water-absorbing during the isolation. In addition, only two elite inbred lines C7-2 and Z58 in China were used in the present study and their SG size was different but comparable, if the SG size of the parental lines were greatly varied, the derived hybrids may varied greatly in seed SG sizes.

We found that the size of seed SG in hybrids was more like that of female parents, especially for large SG population, exhibiting a maternal inheritance trend. This provides some insight on selecting parental inbred lines for breeding maize hybrids with different SG sizes for different applications.

LITERATURE CITED

Alcázar-Alay, S.C., Meireles, M.A.A. 2015. Physicochemical properties, modifications and applications of starches from different botanical sources. J Food Sci Tech Mys. 35: 215-236.

Berger, F., Grini, P.E., Schnittger, A., 2006. Endosperm: an integrator of seed growth and development. Curr Opin Plant Biol. 9, 664–670. https://doi.org/10.1016/j.pbi.2006.09.015.

Cai, C.H., Lin, L.S., Man, J.M., Zhao, L.X., Wang, Z.F., Wei, C.X. 2014. Different structural properties of high-amylose maize starch fractions varying in granule size. J Agr Food Chem. 62: 11711-11721.

Chojkecki, A.J.S., Gale, M.D., Bayliss, M.W. 1986. The number and sizes of starch granules in the wheat endosperm, and their association with grain weight. Annals of Botany. 58: 819-831.

Cui, L., Dong, S., Zhang, J., Liu, P. 2014. Starch granule size distribution and morphogenesis in maize (*Zea mays* L.) grains with different endosperm types. Aust J Crop Sci. 8: 1560-1565.

Ellis, R.P., Cochrane, M.P., Dale, M.F.B., Duffus, C.M., Lynn, A., Morrison, I.M., Prentice, R.D.M., Swanston, J. S., Tiller S.A. 1998. Starch production and industrial use. J Sci Food Agric. 77: 289-311.

Gregorová, E., Pabst, W., Bohačenko, I. 2006. Characterization of different starch types for their application in ceramic processing. J Eur Ceram Soc. 26, 1301-1309.

Hoecker, N., Keller, B., Piepho, H.P., Hochholdinger, F. 2006. Manifestation of heterosis during early maize (Zea mays L.) root development. Theor Appl Genet. 112: 421-429.

Ji, Y., Seetharaman, K., Wong, K., Pollak, L.M., Duvick, S., Jane, J., White, P.J. 2003a. Thermal and structural properties of unusual starches from developmental corn lines. Carbohyd Polym. 51: 439-450.

Ji, Y., Wong, K., Hasjim, J., Pollak, L.M., Duvick, S., Jane, J., White, P.J. 2003b. Structure and function of starch from advanced generations of new corn lines. Carbohyd Polym. 54: 305-319.

Jones, R.J., Schreiber, B.M.N., Roessler, J.A. 1996. Kernel sink capacity in maize: genotypic and maternal regulation. Crop Sci. 36: 301–306.

Kuroiwa, T. 2010. Review of cytological studies on cellular and molecular mechanisms of uniparental (maternal or paternal) inheritance of plastid and mitochondrial genomes induced by active digestion of organelle nuclei (nucleoids). J Plant Res, 123: 207–230.

Li, L., Blanco, M., Jane, J.L. 2007. Physicochemical properties of endosperm and pericarp starches during maize development. Carbohydrate Polym. 67: 630–639.

Li, N., Li, Y. 2015. Maternal control of seed size in plants. J Exp Bot. 66(4): 1087-1097.

Lindeboom, N., Chang, P.R., Tyler, R.T. 2004. Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review. Starch/Stärke. 56: 89-99.

Liu, N., Zhang, Z., Xue, Y., Meng, S., Huang, Y., Li, W., Huang, J., Tang, J. 2018. Identification of quantitative trait loci and candidate genes for maize starch granule size through association mapping. Sci Rep. 8: 14236.

Lu, D.L., Guo, H.F., Lu, W.P. 2011. Effects of sowing date, variety and nitrogen top-dressing at jointing stage on starch granule size distribution of waxy maize. Sci Agric Sin. 44(2): 263–270.

Ma, Y., Cai, C., Wang, J., Sun, D.W. 2006. Enzymatic hydrolysis of corn starch for producing fat mimetics. J Food Eng.73: 297-303.

Matsushima, R., Maekawa, M., Kusano, M., Tomita, K., Kondo, H., Nishimura, H., Crofts, N., Fujita, N., Sakamoto, W. 2016. Amyloplast membrane protein SUBSTANDARD STARCH GRAIN6 controls starch grain size in rice endosperm. Plant Physiol. 170: 1445-1459.

McMaugh, S.J., Thistleton, J.L., Anschaw, E., Luo, J., Konik-Rose, C., Wang, H., Huang, M., Larroque, O., Regina, A., Jobling, S.A., Morell, M.K., Li, Z. 2014. Suppression of starch synthase I expression affects the granule morphology and granule size and fine structure of starch in wheat endosperm. J Exp Bot. 65: 2189-2201.

Ning, F., Wu, X., Zhang, H., Wu, Z., Niu, L., Yang, Hao., Wang, W. 2017. Accumulation profiles of embryonic salt-soluble proteins in maize hybrids and parental lines indicate matroclinous inheritance: A proteomic analysis. Front Plant Sci. 8: 1824.

Niu, L., Ding, H., Hao, R., Liu, H., Wu, X., Hu, X., Wang, W. 2019. A rapid and universal method for isolating starch granules in plant tissues. Plant Cell Environ. 42: 3355-371.

Nordmark, T.S., Ziegler, G.R. 2002. Structural features of non-granular spherulitic maize starch. Carbohydrate Research. 337: 1467-1475.

Paraginski, R.T., Vanier, N.L., Moomand, K., de Oliveira, M., Zavareze Eda, R., Marques e Silva, R., Ferreira, C.D., Elias, M.C. 2014. Characteristics of starch isolated from maize as a function of grain storage temperature. Carbohydrate Polymers. 102: 88-94.

188 Seung, D., Boudet, J., Monroe, J., Schreier, T.B., David, L. C., Abt, M., Lu, K.J., Zanella, M., Zeeman, S.C.
189 2017. Homologs of PROTEIN TARGETING TO STARCH control starch granule initiation in Arabidopsis
190 leaves. Plant Cell, 29: 1657–1677.
191 Seung, D., Smith, A.M. 2019. Starch granule initiation and morphogenesis-progress in Arabidopsis and
192 cereals. J Exp Bot. 70: 771-784.
193 Yu, S., Zhang, F., Li, C., Gilbert, R. G. 2017. Molecular structural differences between maize leaf and
194 endosperm starches. Carbohydrate Polymers, 161: 10–15.
195 Zeeman, S. C., Tiessen, A., Pilling, E., Kato, K. L., Donald, A. M., Smith, A.M. 2002. Starch synthesis in
196 Arabidopsis. Granule synthesis, composition, and structure. Plant Physiology, 129: 516–529.
197 Zeeman, S.C., Kossmann, J., Smith, A.M., 2010. Starch: its metabolism, evolution, and biotechnological
198 modification in plants. Annu Rev Plant Biol. 61: 209-234.
199 Zhang, L., Zhang, J.W., Liu, P., Dong, S.T. 2011. Starch granule size distribution in grains of maize with
200 different starch contents. Scientia Agricultura Sinica. 44: 1596–1602.
201 Zhang, X.D., Chen, Y.F., Zhang, R.H., Zhong, Y.Y., Luo, Y., Xu, S.T., Liu, J.C., Xue, J.Q., Guo, D.W. 2016.
202 Effects of extrusion treatment on physicochemical properties and in vitro digestion of pregelatinized high
203 amylose maize flour. J Cereal Sci. 68: 108-115.
204 Zhao, F., Jing, L., Wang, D., Bao, F., Lu, W., Wang, G. 2018. Grain and starch granule morphology in superior
205 and inferior kernels of maize in response to nitrogen. Sci Rep. 8: 6343.
206 Zi, Y., Shen, H., Dai, S., Ma, X., Ju, W. 2019. Comparison of starch physicochemical properties of wheat
207 cultivars differing in bread- and noodle-making quality. Food Hydrocolloids. 93: 78–86.