Hardness Changes and Endosperm Modification during Sorghum Malting in Grains of Varying Hardness and Malt Quality

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ABSTRACT

This study examined the interaction between sorghum grain hardness and sorghum malt quality in terms of diastatic power and free amino nitrogen with endosperm modification during malting. The changes in kernel hardness during malting of four commercial sorghum cultivars of differing quality in terms of endosperm texture and potential malt quality were measured using tests for hardness and density, and endosperm modification was followed by scanning electron microscopy. The general pattern of modification during sorghum malting was confirmed to start at the ensdosperm-scutellum interface, then into the floury endosperm towards the kernel distal end. Significantly, a cultivar of intermediate hardness and low malting quality remained harder and modified more slowly than a harder cultivar of high malting quality. It appears that intrinsic grain hardness and malt amylase and protease activity both affect malt hardness and endosperm modification, but amylase and protease activity have a greater effect due to their degradation of endosperm starch and protein. Of the hardness and density tests studied, the Tangential Abrasive Dehulling Device (TADD) gave the best measure of hardness throughout malting; maximum range 24% to 100% kernel removed over 5 days malting. Also, the data agreed with the observed malt modification rates. Thus, the TADD may have application as a simple and rapid test for estimating sorghum malt quality.

INTRODUCTION

Sorghum malt is widely used as a major functional component of traditional African beers, lagers and stouts, non-alcoholic malt beverages and porridges (Taylor and Emmambux 2008). During the sorghum malting process the starchy endosperm is modified. The pattern of sorghum endosperm modification during malting was comprehensively studied by Glennie et al (1983). They found that sorghum endosperm modification is characterized by degradation of the starch granules, protein matrix and protein bodies by endogenous hydrolytic enzymes. Modification starts at the endosperm-scutellum interface into the floury endosperm and lastly the corneous endosperm. Within cells, the glutelin endosperm protein matrix is degraded first, while starch granules and kafirin containing protein bodies degraded at the same time. Degradation of starch granules was evidenced by pitting of the granules. Importantly, in sorghum, the starchy endosperm cell walls remain intact after malting (Glennie et al 1983; Glennie 1984; Palmer 1991), unlike in barley malting where they are degraded (Morrall and Briggs1978, EtokAkpan and Palmer 1990).

In barley malting the extent of endosperm modification is related to malt quality and modification can be measured in terms of friability, the tendency of the modified malt to break into fine particles when milled under specific conditions (reviewed by Briggs 1998). Using instruments such as the Friabilimeter (Fox et al 2001) and the Single Kernel Hardness System (SKCS) (Nagamine et al, 2009), it has been shown that the friability of barley malt is correlated with several malt quality parameters including malt extract.

Because of its importance, endosperm modification in barley malting is the most researched among cereals. Barley malt hardness has been found to decrease by the second day of malting (Osborne and Anderssen 2003; Osborne et al 2005). The decrease was attributed to the

softening of the grain outer layers during steeping and loss of cellular structure, reduced dry matter (malting loss), loss of kernel orientation and endosperm collapse. Brennan et al (1997) showed that the malt modification in good quality barley malting cultivars was characterized by protein degradation from the sub-aleurone layer towards the inner endosperm, a different pattern to that observed in sorghum (Glennie et al. 1983). Protein degradation in the inner endosperm occurred after more than four days of malting. Starch granule degradation, was evidenced by pitting and partial concentric shell destruction after almost six days. The endosperm cell walls were no longer visible after this malting period.

Studies (Psota et al 2007, Vejrazka et al 2008) found a relationship between the duration of barley malting and hardness as a predictor of the malting quality of barley. Psota et al (2007) showed that grain hardness adversely affected accessibility of hydrolytic enzymes to the starchy endosperm in barley. In sorghum, grain hardness is an important parameter of grain quality (Taylor and Duodu 2009). Hard sorghum grains are desired for dry milling as they produce a high yield of pure endosperm grits (Munck 1995). However, the relationship between sorghum grain hardness and grain modification during malting is not known. Since the same sorghum cultivar is often used for both dry milling and malting, as is the case in Southern Africa, this study sought to qualitatively examine the interaction between sorghum grain hardness and malt quality in terms of diastatic and free amino nitrogen with endosperm modification during malting.

MATERIALS AND METHODS

Grain Samples

Four commercial hybrid sorghum cultivars grown in a controlled field trial at Potchefstroom, South Africa, in the 2008-2009 season, under dryland conditions, were used in this study. The grain was field dried and harvested at less than 14% moisture content. The cultivars were selected for hardness according to the percentage of kernel removed by the Tangential Abrasive Dehulling Device (TADD), which was used as described by Chiremba et al (2011). These authors showed the TADD was very suitable method for determining sorghum grain hardness.. The proportion of corneous endosperm was estimated according to ICC Standard 176 (ICC 2011) and the cultivars classified as corneous, intermediate or floury. The sorghum cultivars were PAN 8247 and PAN 8901 (hard/corneous, red non-tannin), PAN 8648, (intermediate, white tan-plant non-tannin) and PAN 8625 (soft/floury, red condensed tannin). The sorghum cultivars were comprised of three malting classes in terms of Diastatic Power (DP) (joint alpha- and beta-amylase activity). Cultivars PAN 8247 and PAN 8901 were of the GM class (high DP, non-tannin), PAN 8648, GL class (low DP, non-tannin) and PAN 8625, GH class (high DP, tannin) and assigned to these classes by the South African Department of Agriculture (1994). The two sorghum cultivars with similar characteristics, PAN 8247 and PAN 8901, were used to confirm the effect of malting on grain hardness and density.

Grain and Malt Analyses

Grain Germinative Energy was measured at 72 hr germination by ICC Standard 174 (ICC 2011). Grain was malted at the laboratory scale according to Dewar et al (1995), with modifications. Cleaned grain (500 g) were steeped at 25^{o}C for 24 hr and then malted for five days also at 25^{o}C. The cultivar PAN 8625 was soaked in a 0.2% (w/v) NaOH solution for 4 h prior to steeping, in order to inactivate the condensed tannins. On each day of malting, a portion of malt was sampled, weighed and then dried at 50°C for 24 hr in a forced-draught oven to a shelf-stable moisture content of approximately 8%.

Diastatic Power (DP) of the 5 day malts (after steeping) was measured according to South African Bureau of Standards Method 235 (SABS 1970), modified as described by Dewar et al (1995) using peptone extracts and expressed as Sorghum Diastatic Units (SDU/g). Malt free amino nitrogen (FAN) was determined according to the European Brewery Convention ninhydrin method 4.10 (EBC 1998), modified as described by Dewar et al (1995) and expressed as mg FAN/100 g malt.

Raw grain and malt hardness were measured using the TADD by decorticating 50 g samples for 5 min (Gomez et al 1997). Single kernel hardness was measured with a SKCS 4100 (Perten Instruments, Huddinge, Sweden) (Bean et al 2006). One thousand kernel weigh (TKW) was determined by weighing 1000 sound grain or malt kernels of a representative sample.

Grain and malt density were estimated using a floatation test, where 50 sound kernels were immersed in a clean solution of sodium nitrate with a specific gravity of 1.275 g/cm^3 and expressed as percent floaters (Paulsen et al 2003). Specific density was determined using a gas pycnometer (Model MUP-1 S/N 232, Quantachrome, Syosset, NY). All malt quality tests were performed on whole malt (including external roots and shoots).

Scanning Electron Microscopy (SEM)

Sorghum grains and malts of PAN 8247 (the hardest grain as measured by the TADD, Table I), PAN 8648 (intermediate) and PAN 8625 (soft) were immersed in liquid nitrogen at -196°C. The frozen samples were cut longitudinally through the germ with a sharp blade and mounted on aluminum stubs using adhesive tape. The mounted samples were sputter coated with gold and then viewed using a Zeiss Evo LS15 scanning electron microscope (Carl Zeiss, Oberkochem, Germany) operated at an acceleration voltage of 8 kV.

Statistical analyses

Sorghum grain hardness, malt hardness and malt quality tests were performed in triplicate. Fischer's least significant difference (LSD) test was used to compare means. Calculations were performed using Statgraphics Centurion XV (StatPoint, Herndon, VA).

RESULTS AND DISCUSSION

Germinative Energy was at least 90% and similar for four cultivars investigated (Table I). Thus, as the sorghums germinated uniformly they were suitable to be malted (Morrall et al 1986), and to compare their malting qualities. Water uptake after steeping was substantially lower in the hard cultivars PAN 8247 and PAN 8901, probably due to these cultivars having a more corneous endosperm,as seen with PAN 8247 (Fig 1). Malting loss was the lowest in PAN 8648 (intermediate), suggesting least endosperm hydrolysis. Also, as would be expected, as it is not a malting class cultivar, PAN 8648 had the lowest malt quality in terms of DP (amylase activity) and FAN.

Table II shows the effect of malting on the hardness of these cultivars as assessed using the various hardness and density tests over the five day malting period. Of the tests applied, only the TADD quantified very large changes in kernel hardness over the 5 days of malting, from 31.8 and 23.9% to 96.9 and 97.78% kernel removal for the two hard cultivars PAN 8901 and PAN 8247, respectively, and 63.2% to 99.5% for the soft cultivar PAN 8625. Of note was that by 2

days malting the two hard cultivars were significantly softer ($p < 0.05$) than the intermediate PAN 8648 and this cultivar remained somewhat harder up until day 5. As stated, unlike the other three cultivars, PAN 8648 is not a malting class sorghum and had lower DP and FAN (Table I). Also of note was that by day 3 the two harder grains were as soft as the soft cultivar PAN 8625.

The SKCS system was less sensitive than the TADD in that with raw grain only the soft cultivar PAN 8625 was significantly softer ($p<0.05$). Notably, however, the SKCS system, like the TADD, also indicated that after 2 days malting, the intermediate cultivar PAN 8648 was significantly harder ($p<0.05$) than the two hard cultivars. However, by 3 days malting all the grains were too brittle to be measured by the SKCS. The SKCS measures kernel hardness by a response to crushing (Osborne and Anderssen 2003). The initial crush response is a factor of the pericarp and aleurone layer and finally, compression of the endosperm. It is probable that when the SKCS was applied the modified kernel endosperm collapsed and the kernel shape flattened, hence the malt kernels could not be evaluated. In contrast to the SKCS, the TADD progressively removes the outer layers of the grains as they roll around in the cylinders mounted on an abrasive carborundum disc (Taylor and Duodu 2009); hence, the harder the grain the slower the material is abraded off. The effectiveness of the TADD could be attributed to its relatively gently abrading action unlike the SKCS where compression is applied. Thus, even when the soft modified malt was analyzed the unmodified pieces of grain were not crushed to powder.

TKW was not discriminating with respect to the hardness of the different malts, although malting substantially reduced TKW. The floaters grain density test was too crude to detect differences between the cultivars as floaters increased to >90% in all cultivars by one day malting. This dramatic increase was probably due to the creation of internal air spaces as a result

of hydrolysis of endosperm cell contents (Glennie et al 1983). True density as measured by gas pynometry also revealed a fairly substantial decrease in grain density between 1 day malted and raw grain. Thereafter, density decreased slowly during the course of malting. However, there was no significant difference between the cultivars, probably because the reduction in density over the entire five days of malting was small, only some 4-9%.

SEM of kernel longitudinal sections (Fig 1) gives an overview of the floury and corneous endosperm and the general structural changes with malting time. The floury endosperm area of PAN 8625 grain (soft) (Fig 1G) was larger than that of PAN 8247 (hard) and PAN 8648 (intermediate), which is related to its greater softness as measured by the TADD and SKCS, and lower grain density as measured by the floatation test (Table II). On days 1 and 3 of malting, all the cultivars all showed evidence of starch degradation at the endosperm-scutellum interface (Fig 1), confirming that modification starts in this region into the floury endosperm (Glennie et al 1983). Considerable reduction in malt hardness as measured by the TADD and SKCS occurred during this period (Table II).

SEM of proximal sections of the grain showed that in the floury endosperm at the near the endosperm-scutellum interface by day 3 of malting there was a network of cell walls devoid of starch granules in all the cultivars (Fig 2C,F,I). This endosperm modification can be attributed to enzymatic hydrolysis of starch granules, protein bodies and the protein matrix, as observed by Glennie et al (1983), resulting in air spaces and the observed considerable reduction in hardness and decrease in density (Table (II). It is notable that on Day 3 of malting the level of endosperm modification in PAN 8648 (Fig 2F) was less than in the other two cultivars, as evidenced by the denser appearance of the floury endosperm. This explains the higher hardness of PAN 8648 at 3 days of malting as measured by the TADD (Table II). It also suggests that the

lower level of endosperm modification was related to the lower levels of amylase and protease activity (the latter indicated by the lower level of FAN, the product of proteolysis) in its malt (Table I).

SEM of the corneous endosperm in the grain middle region (Fig 3) showed little evidence of modification by day 3 of malting in any of the cultivars, in contrast to the situation in the floury endosperm after 3 days (Figs 1 and 2). The corneous endosperm cells of PAN 8247 (hard and PAN 6525 (soft) (Fig 2C,I), however, showed some separation, indicative of slight modification. This is in contrast to PAN 8648 (intermediate) (Fig 2F) where the individual cells could not be seen, indicating no modification. This observation agrees with the fact that PAN 8648 was significantly harder at this stage of malting (Table II) and its malt had lower DP and FAN (Table I). SEM of the distal region (Fig 4) showed that there were no structural changes in the kernel, with the floury endosperm cells appearing in PAN 8625 (soft) appearing to be intact and full of starch granules (Fig 4I).

However, high magnification SEM of cultivars malted for 5 days showed that the starch granules at the distal end and middle sections of PAN 8625 were slightly degraded (Fig 5C,F), as indicated by pitting. Some starch granules in cells at the distal end of PAN 8247 (hard) were free from the surrounding protein matrix (Fig 5A). This agrees with observations of 7 day malted sorghum where the protein bodies were detached from the starch granules (Correia et al 2008). In contrast, those in PAN 8648 (intermediate) were still embedded (Fig 5B), indicating slower modification in this cultivar, in agreement with it being generally harder during malting (Table II).

SEM of the proximal end of the 5 day malted cultivars revealed extensive starch granule degradation in PAN 8247 (hard) (Fig 5G), with endosperm cell wall being present. This also agrees with the observations of Correia et al (2008) of 7 day germinated sorghum where the starch granules were strongly attacked and eroded. PAN 8648 (intermediate) showed less starch degradation (Fig 5H), again indicative of its lower amylase activity and malting loss (Table I) and generally harder nature during malting (Table II). In contrast, starch granules at the proximal end of PAN 8625 (soft) were almost totally degraded (Fig 5I), but the endosperm cell wall was present, although very torn. Cell wall tearing is a consequence of the partial enzymatic degradation of the sorghum endosperm cell walls, which occurs during malting (Palmer 1991) and physical damage during sample preparation for SEM. Physical damage is likely to occur considering that the endosperm cell contents (starch granules, protein bodies and matrix bound to the cell wall), which provided support for the cell walls, are also degraded during malting (Glennie 1984).

One of the reasons for the persistence of the sorghum endosperm cell walls during malting is that sorghum glucuronoarabinoxylans are highly substituted, in comparison to those of barley (Verbruggen et al 1998). Ferulic and diferulic acids are cross linked to glucuronoarabinoxylan chains, and thus could hinder action of the xylanases, arabinofuranosidases and glucuronidases and other enzymes that hydrolyze the glucuronoarabinoxylans (Verbruggen et al 1998). The interaction of ferulic acid with endosperm cell walls could play a role in maintaining the integrity of the sorghum cell walls during malting ultimately contributing to malt hardness (Chiremba et al 2012).

CONCLUSIONS

The general pattern of endosperm modification during sorghum malting was as described by Glennie et al (1983). However, the extent of modification differs substantially between cultivars. It appears that there is an interplay of two factors: intrinsic grain hardness and malt amylase and protease activity, both of which affect sorghum malt hardness and endosperm modification. PAN 8648, a cultivar of intermediate hardness and with low malt quality with respect to DP and FAN remained harder and less modified during malting than PAN 8247, a hard cultivar with high DP and FAN. It therefore appears that high malt amylase and protease activity because of their impact on starch granule and protein matrix degradation, have a greater effect on sorghum malt hardness than intrinsic grain hardness. Of the hardness and density tests studied, the TADD gave the best measure of kernel hardness throughout malting, and the data agreed with the observed differences in malt modification rate between cultivars. Thus, the TADD may have application as a simple and rapid test for estimating sorghum malt quality.

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TABLE I

Malting Properties of Sorghum Cultivars Varying in Hardness

Cultivar and Hardness	Germinative Energy (%, 72 hr)	Water Uptake During Steeping[a] (%)	Five-day Malting Loss[a,b] (%)	Five-day Malt Diastatic Power[b] (SDU/g, db)	Five-day Malt Free Amino Nitrogen[b] (mg/100 g, db)
PAN 8901 (Hard)	91.8 a (3.7)	33.3 b (3.0)	17.4 ab (0.2)	44.4 a (1.7)	214 ab (12)
PAN 8247 (Hard)	92.3 a (5.7)	33.8 b (1.6)	17.3 ab (0.5)	41.3 b (0.5)	206 b (4)
PAN 8648 (W) (Intermediate)	92.5 a (2.5)	41.3 a (6.9)	16.3 c (0.2)	25.2 c (0.5)	184 c (13)
PAN 8625 (T) (Soft)	93.1 a (4.5)	40.7 a (1.5)	17.9 a (0.4)	47.0 a (1.3)	236 a (16)
Mean	92.4 (4.1)	37.2 (5.0)	17.2 (0.7)	39.4 (9.3)	210 (11)

(T), Condensed tannin sorghum; (W), White tan-plant, non-tannin sorghum

[a] Percentage of original grain weight, as is

[b] Results of whole malt including external roots and shoots

Figures in parentheses are standard deviations

Different letters in the same column denote significant differences at $p < 0.05$

n=3

TABLE II

Effect of Malting Time on the Hardness and Density of Sorghum Cultivars

Cultivar and Hardness	Malting Time (Days)	TADD Hardness (% Kernel Removed)	SKCS (Hardness Index)	TKW (g)	Floaters (%)	Gas Pycnometer (g/cm^3)
PAN 8901	0	31.8k (1.3)	69.6ab (1.8)	29.4a (1.9)	18.7d (1.2)	1.37a (0.00)
(Hard)	1	50.3i (1.2)	58.1bc (1.5)	27.3abc (0.7)	93.0b (4.2)	1.32bcd (0.01)
	2	64.4g (2.4)	44.7d (4.3)	26.1a-d (0.4)	95.0b (7.1)	1.32bcd (0.01)
	3	83.4e (1.2)	ND	23.4b-g (0.7)	100.0a	1.31bcd (0.01)
	4	92.2bcd (1.1)	ND	22.4d-h (0.2)	100.0a	1.31bcd (0.00)
	5	96.9ab (0.5)	ND	20.3fgh (1.1)	100.0a	1.28cde(0.00)
PAN 8247	0	23.9l (0.9)	73.6a (0.8)	28.0ab (0.4)	6.00e (2.0)	1.37a (0.01)
(Hard)	1	48.9j (0.4)	60.2b (0.5)	26.8a-d (0.9)	91.0b (1.4)	1.32bcd (0.00)
	2	66.2g (1.8)	42.8d (0.4)	26.1a-d (0.9)	100.0a	1.29e-i (0.01)
	3	87.3ef (0.6)	ND	22.5c-h (0.7)	100.0a	1.27c-g (0.00)
	4	96.5abc (0.3)	ND	20.4fgh (1.9)	100.0a	1.25g-j (0.01)
	5	97.7a (0.3)	ND	19.1gh (0.2)	100.0a	1.24h-k (0.01)
PAN 8648)	0	40.9j (1.7)	75.5a (2.7)	26.8a-d (0.4)	6.67e (1.2)	1.36ab (0.01)
(W)	1	45.4 ij (1.9)	63.1b (1.3)	25.6a-d (0.2)	91.0b (1.4)	1.32bcd (0.00)
(Intermediate)	2	58.6h (1.6)	54.4c (0.9)	24.5b-f (1.8)	100.0 a	1.31c-f (0.01)
	3	77.9f (0.2)	ND	22.3d-h (0.7)	100.0 a	1.30f-j (0.01)
	4	82.2fg(1.5)	ND	21.0e-h (1.4)	100.0 a	1.29cd (0.00)
	5	91.4cd (0.3)	ND	18.8gh (0.7)	100.0 a	1.26c-g (0.01)
PAN 8625	0	63.2i (2.4)	57.7bc (2.7)	27.3abc (1.0)	34.7c (3.1	1.33abc (0.00)
(T)	1	63.2gh (1.7)	57.0bc (0.6)	24.5b-f (0.7)	95.0b (7.1)	1.30c-f (0.00)
(Soft)	2	77.8f (0.5)	41.9d (0.5)	24.0b-f (1.4)	100.0a	1.27f-I (0.01)
	3	89.3e (1.0)	ND	20.0fgh (0.4)	100.0a	1.23ijk (0.00)
	4	95.0abc (0.0)	ND	19.6fgh (0.7)	100.0a	1.22jk (0.00)
	5	99.5a (0.1)	ND	18.4h (0.2)	100.0a	1.21j (0.00)

ND, Not determined as most kernels rejected by the SKCS
(T), Condensed tannin sorghum; (W), White tan-plant, non-tannin sorghum
TKW, Thousand kernel weight, as is basis
Figures in parentheses are standard deviations
Different letters in the same column denote significant differences at $p < 0.05$
Day 0, raw grain; Days 1-5; malting time after steeping
n=3

LEGENDS TO FIGURES

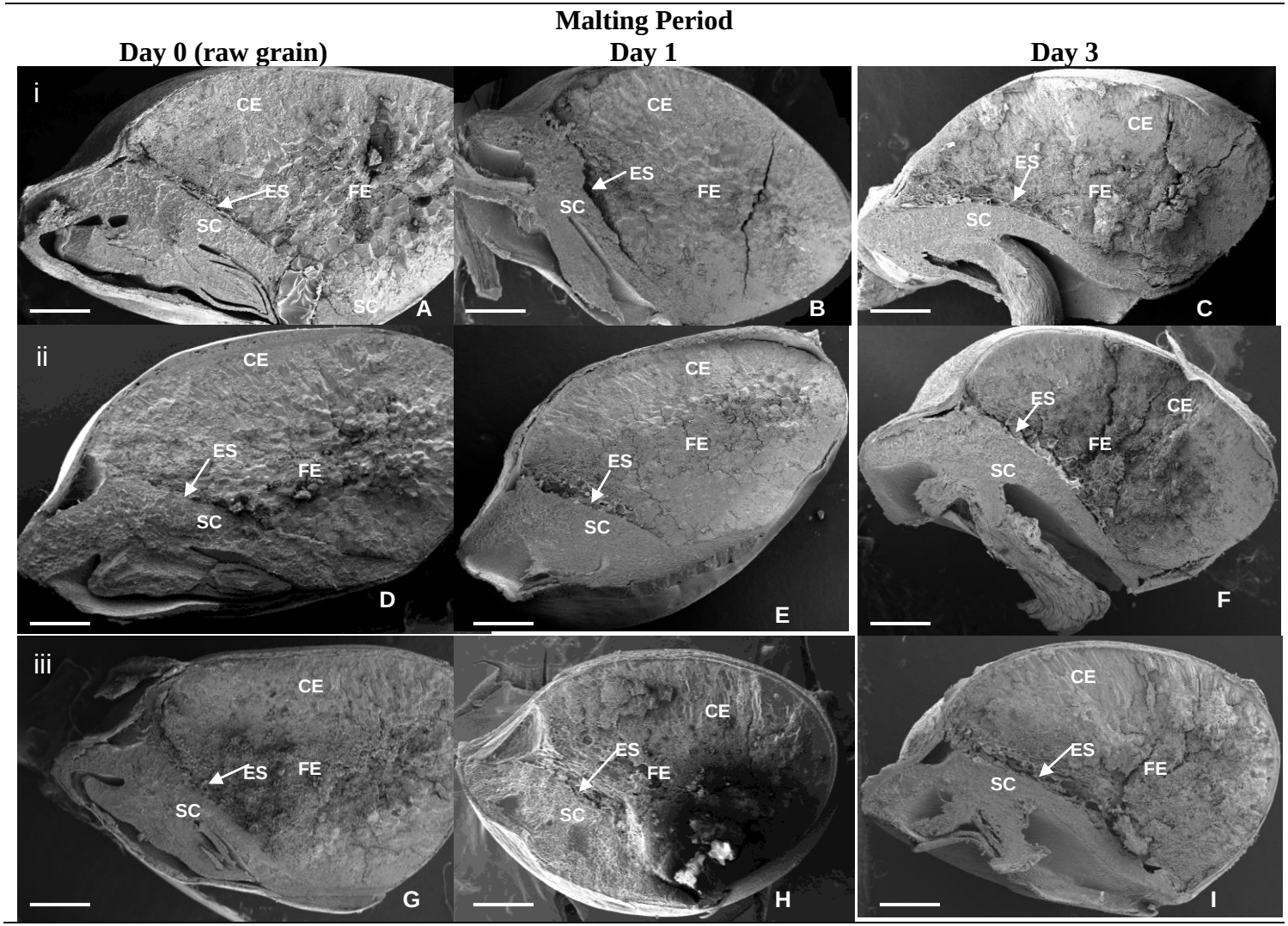
Fig 1. SEM of longitudinal sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), corneous endosperm (CE), floury endosperm (FE), scutellum (SC) and endosperm degradation at interface with scutellum (ES). Bar is 1 mm.

Fig 2. SEM of proximal sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), floury endosperm (FE), network of cell wall devoid of starch granules (CWd) and scutellum (SC). Bar is 200 µm.

Fig 3. SEM of middle sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), the pericarp (P), corneous endosperm (CE) and testa (T). Bar is 200 µm.

Fig 4. SEM of distal sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), pericarp (P), corneous endosperm (CE), floury endosperm (FE) and testa (T). Bar is 200 µm.

Fig 5. SEM of (i) distal, (ii) middle and (iii) proximal sections of sorghum grain of different hardness that had been malted for up to 5 days following steeping. PAN 8247 (hard), PAN 8648 (white tan-plant, intermediate), PAN 8625 (condensed tannin, soft), smooth cell wall (CWs), torn cell walls (CWt) intact starch granules (SG), degraded starch granules (SGd) and protein bodies (PB). Bar is 10 µm.



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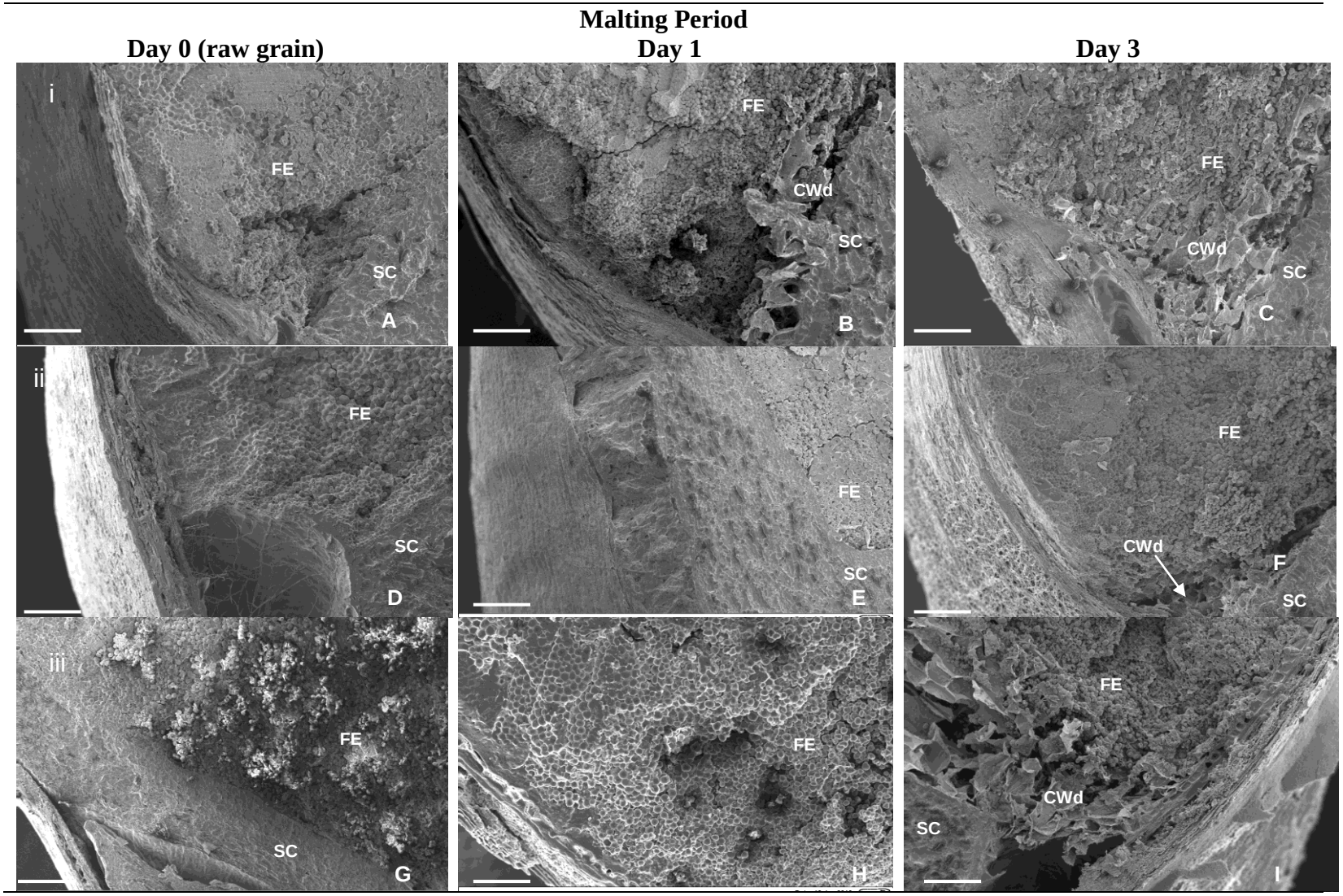

Malting Period
Day 0 (raw grain)
Day 1
Day 3
i
FE
SC
A
FE
CWd
SC
B
FE
CWd
SC
C
ii
FE
SC
D
FE
SC
E
FE
CWd
F
SC
iii
FE
SC
G
FE
H
FE
CWd
SC
I

Fig 2. SEM of proximal sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), floury endosperm (FE), network of cell wall devoid of starch granules (CWd) and scutellum (SC). Bar is 200 µm.

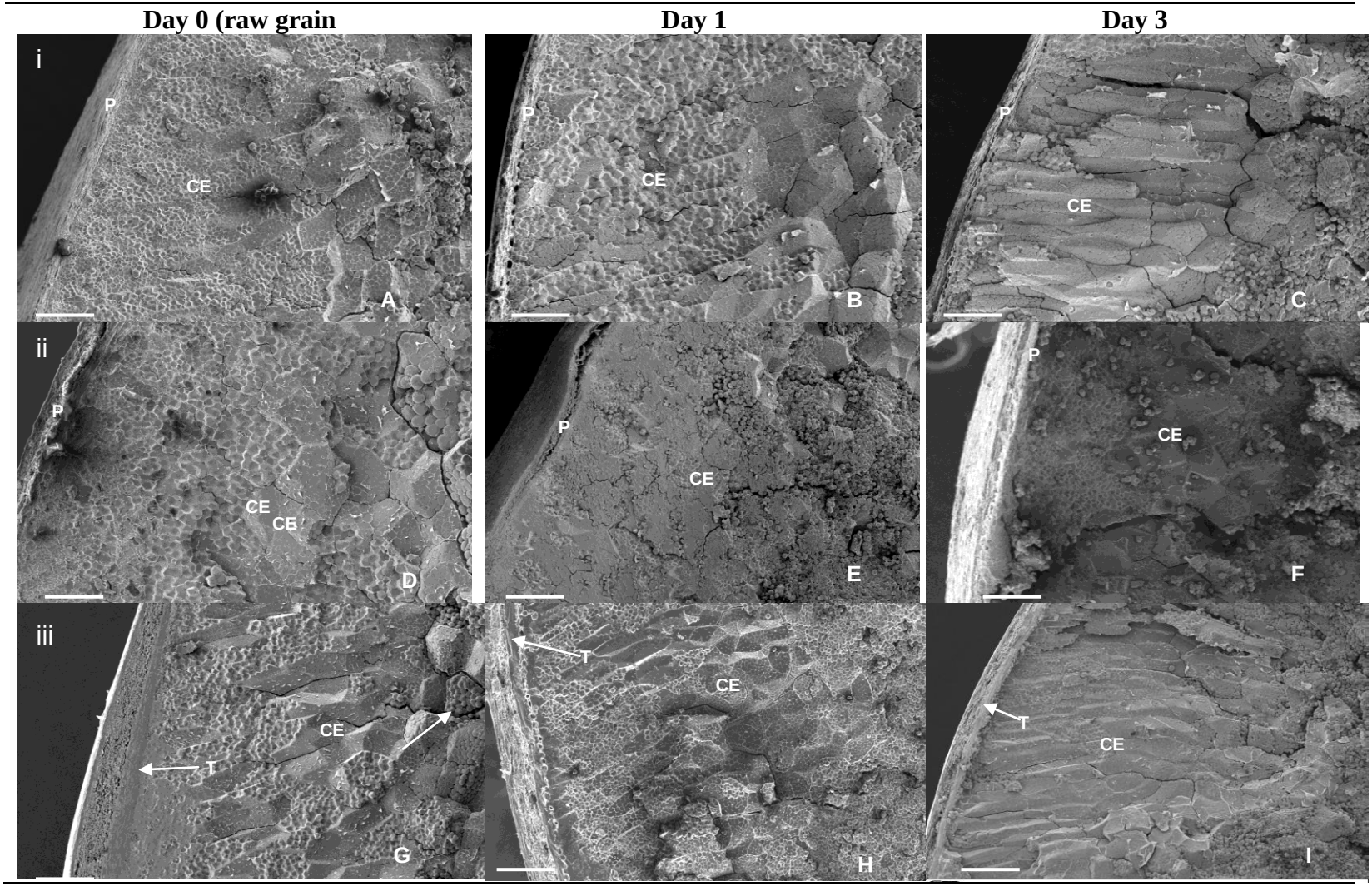
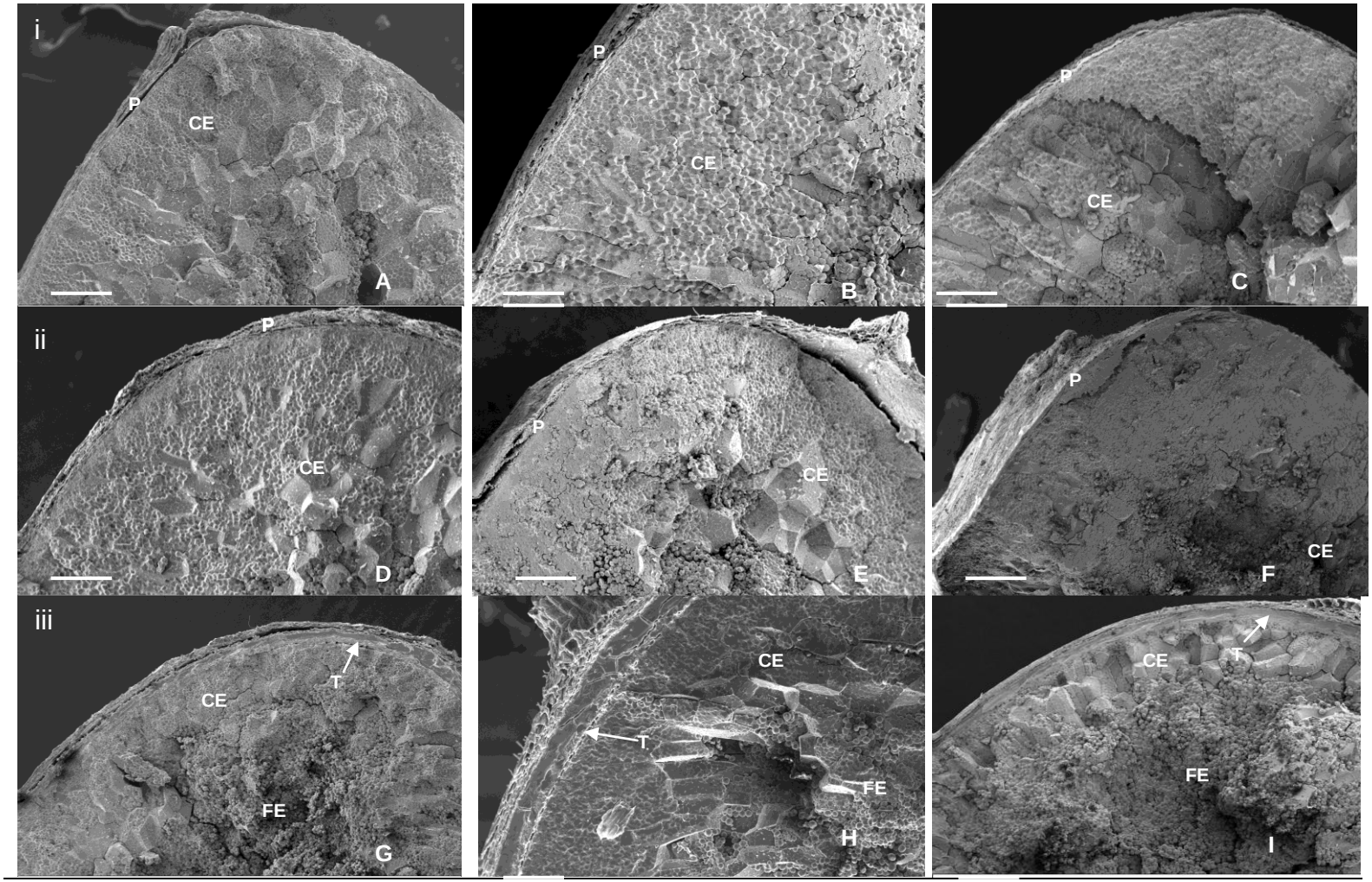

Day 0 (raw grain
Day 1
Day 3
i
ii
iii
P
CE
T
A
B
C
D
E
F
G
H
I

Fig 3. SEM of middle sections of sorghum grain of different hardness that had been malted for up to 3 days following steeping. (i) PAN 8247 (hard), (ii) PAN 8648 (white tan-plant, intermediate), (iii) PAN 8625 (condensed tannin, soft), the pericarp (P), corneous endosperm (CE) and testa (T). Bar is 200 μm.

Malting Period

Day 0 (raw grain) **Day 1** **Day 3**



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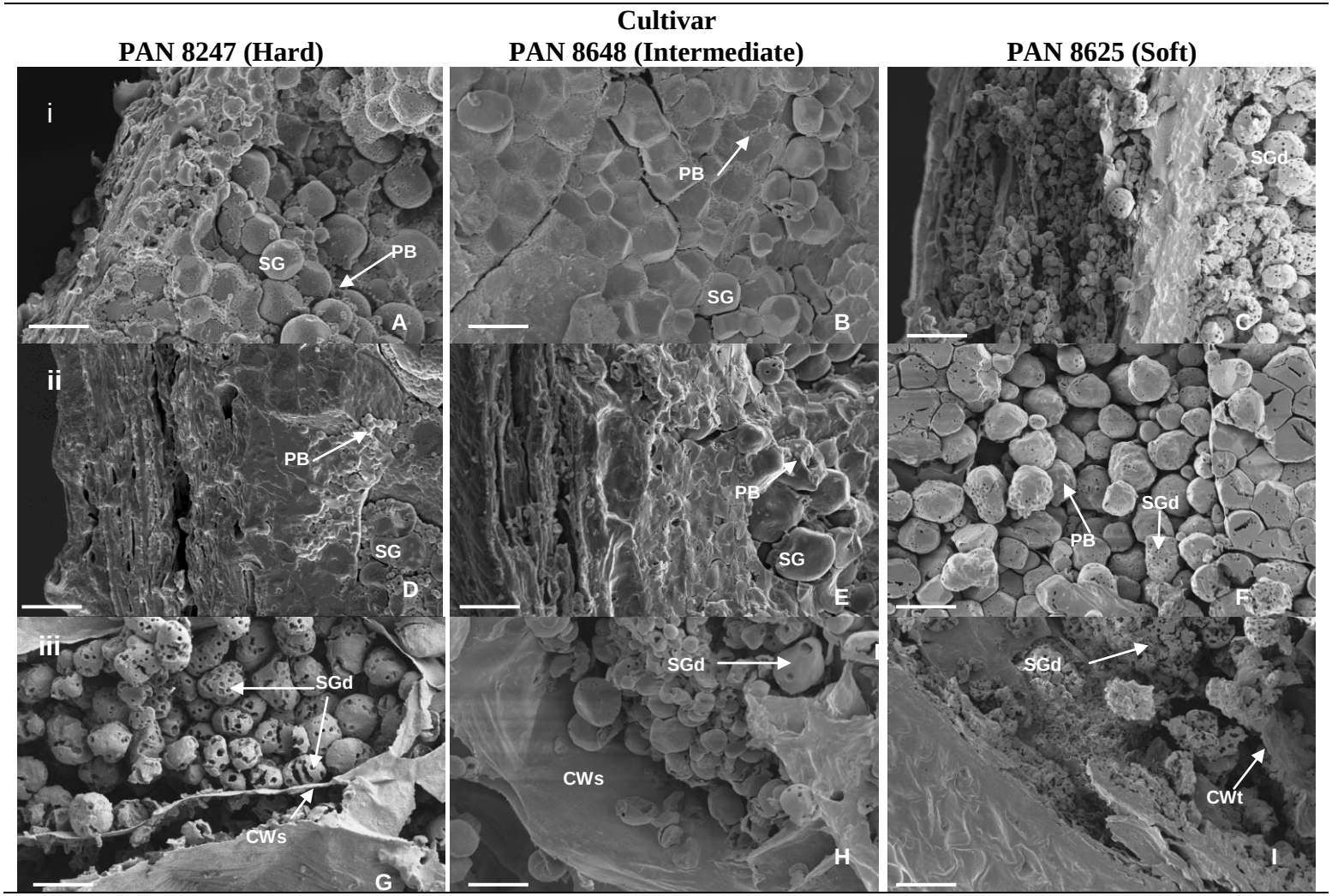

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