Fiber Quality Variability Within a Plant

Neha Kothari

International Textile Center, Texas Tech University, Lubbock TX 79407

Tel: 806-747-3790

Email: neha.kothari@ttu.edu

Noureddine Abidi (corresponding author)

International Textile Center and Department of Plant and Soil Science, Texas Tech University, Lubbock TX 79407

Tel: 806-747-3790

Email: n.abidi@ttu.edu

Eric Hequet

International Textile Center and Department of Plant and Soil Science, Texas Tech University, Lubbock TX 79407

Tel: 806-747-3790

Email: eric.hequet@ttu.edu

Thea Wilkins

Dept. of Plant and Soil Science, Texas Tech University, Lubbock, TX

Phone**:** 806-742-3722

Email: thea.wilkins@ttu.edu

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ABSTRACT

Our primary goal in this research is to study the spatial distribution of fiber maturity and length within a cotton plant. It is of the utmost importance for geneticists and biotechnologists to realize that the fibers sampled for analysis and testing should be representative of the plant or genotype under study. Two genotypes were selected, namely TM-1 and *im*. TM-1 is a wild type cotton and the genotype *im* is an isogenic mutant of TM-1, which has been reported to be genetically immature. Twenty plants each, of TM-1 and *im* were tagged in the field and harvested. Cotton bolls were identified by fructifer branch number and by position within each branch. Cotton samples collected from different positions in the plant were hand-ginned to prevent fiber breakage. Advanced Fiber Information System and High Volume Instrument were used to measure length, maturity ratio and their distribution as well as other pertinent fiber properties. The results obtained show that there is a large variability for both, length and maturity distributions when progressing from the bottom of the plant to the top. In addition, the extent of the fiber quality variation appears to be genotype specific. These results demonstrate that appropriate sampling protocols need to be developed for single plant testing (cotton breeding for example).

Keywords: HVI, AFIS, Maturity, Micronaire, TM-1, *im*

INTRODUCTION

The cotton plant growth habits lead to tremendous variation in fiber quality within genotypes, within plant, and within a boll. This variability is, in general caused by an interplay of various metabolic processes and regulatory mechanisms. In addition, it has been reported that soil pH and organic content could result in variation in fiber yield and quality (Johnson, et al. 2002, Elms, et al. 2001). Also, Moisture availability, irrigation timing and level, soil fertility, to name a few are also a cause of fiber quality variability. Under controlled growth conditions, most fibers develop well. However, even under the best growth conditions within a boll, some fibers will be poorly developed (Wilkins, et al., 1999).

The environment plays an important role in variability of cotton fiber quality. It has been shown that cotton fiber quality changes with boll location within the plant and planting dates (Davidonis et al., 2004). Krieg (2005) reported that temperature and growth environment also lead to variation in cotton fiber quality. Exposure to sunlight (UV radiation) affects fiber quality. Ultra-violet radiation causes breakage of cellulose chains, leading to weaker fibers (Morton and Hearle, 1997). Lint from open bolls can be negatively affected if exposed to rain before being harvested.

Besides genotype and environmental conditions affecting fiber quality, harvesting, ginning, and processing methods can also alter fiber quality parameters of cotton (MacAlister et al., 2005). It is known that harvesting methods impact fiber quality and length distributions (Behery, 1993). Cotton stripping tends to generate higher short fiber content than cotton picking because stripper cotton contains more foreign matter than picked cotton (El Mogahzy and Chewning, 2001). Therefore, stripped cotton needs additional lint cleaning at the gin. This additional mechanical stress leads to more fiber

breakage and, thus, increases the short fiber content. Ginning techniques influence fiber length distributions. It is known that saw ginning tends to break more fibers than roller ginning while hand ginning tends to break less fibers than roller ginning (Robert et al., 2000). Hand ginning is not an industrial process and it is used only in research. At an industrial level, saw ginning is widely used and this procedure aids in the proper mixing of the fibers to some extent. Roller ginning is less effective in mixing the lint. In addition to hand ginning in research programs, saw ginning and roller ginning are used. The small laboratory gins almost never lead to proper mixing, hence it is very important (before fiber testing) to use a blender to mix the lint of the ginned samples, because of the high variability of fiber quality seen within a plant.

Ginning leads to fiber breakage which is closely related to individual fiber strength and therefore to maturity. Fiber maturity is a very important and critical fiber quality trait in the cotton industry (Basra and Malik, 1984). Immature fibers tend to break easily and generate high short fiber content, thereby affecting fiber length (Hequet et al. 2000). Wilkins et al. (1999) reported that individual fibers in the same boll of cotton show variation in the cell wall thickness. Depending on where a sample is picked from the plant and the way it is processed, it can give different fiber quality measurements.

In this paper we report on the variability of fiber properties within a cotton plant for two extremely different genotypes, TM-1 and *im*. TM-1 is the wild type cotton and *im* is its isogenic mutant known to grow genetically immature fibers (Kohel, 1990).

MATERIALS AND METHODS

Materials

TM-1 (Texas Marker-1, identified as TM) and *im* (immature cotton) were grown in the green-house (located at TG Trees, Lubbock, TX) and in the field (Texas Tech

Quaker Farm, Lubbock, TX) with randomized complete block design. Each plot had about 40 plants. Planting was done in the green-house on July 20, 2006. Green-house growth protocol was 30°C/20°C for day/night routine in optimal conditions with 5 gallon pots and 3 plants/pot. Drip irrigation system was used. In the field, planting was done on June 8, 2006.

Harvesting

To study the variability of the fiber quality (especially maturity and length) we harvested cotton bolls at different positions within the plant. These samples are called the plant 'mapping' samples. For this purpose, twenty plants were tagged for each genotype. Opened bolls were collected from different fruiting branches and boll positions, and samples were identified as TM-x-y or *im*-x-y, where x refers to the fructifer branch number and y refers to the boll potion within that branch. For example, TM-8-1 would mean genotype TM fructifer branch 8 and boll position 1. Branch numbering started from the bottom (just after the last vegetative branch) of the plant to the top. Bolls were harvested starting October 16, 2006 and by November 15, 2006 all the bolls were harvested.

From the remaining 20 plants in the field for each plot, a combined 'average sample' was harvested. An 'average sample' was obtained by harvesting all the bolls from 20 plants for each cultivar (for each of the replicates), roller ginning the bolls and then mixing them with a laboratory blender. Only two replications were harvested in the green-house since the third one had been lost due to poor yield. Harvesting for the green-house average samples was done on January 25, 2007.

Ginning and blending

Bolls harvested at different positions (mapping samples) in the plant were hand-ginned. The lint from identical branches and boll positions were combined for each genotype to generate sufficient lint for testing. We collected a set of twelve boll positions for both TM-1 and *im.*

The average samples from the field and green-house were machine ginned with a small tabletop roller gin. A mechanical cotton blender was used to mix the cotton to reduce the effects of within-sample variability.

HVI and AFIS testing

The samples were conditioned in the laboratory at 65% relative humidity and 21°C (standard laboratory conditions) for at least 48 hours before testing. Fiber testing was done at the International Textile Center, Texas Tech University, Lubbock. The instruments employed were, *Uster*® HVI 900A (High Volume Instrument) and *Uster*® AFIS PRO (Advanced Fiber Information System). A module test 1,10 (1 micronaire test and 10 length and strength tests) with the HVI was done on the combined average samples from field and green-house. AFIS testing was performed on all the samples. For AFIS testing, 5,000 fibers were counted and three replications were performed (total of 15,000 fibers analyzed). AFIS length and maturity distributions were plotted.

RESULTS AND DISCUSSION

Fiber quality Analysis of average samples from the green-house and the field

HVI data analysis: Tables 1-a and 1-b show the HVI results for TM-1 and *im* in green-house and field conditions. A micronaire of 2.0 was observed for *im*, in the green-house as well as in the field, which is the lowest reading that the instrument measures. TM-1 had a micronaire of 3.2 when grown in the green-house, and 2.8 when grown in the field revealing that even the wild-type cotton was somewhat immature that year. This is

consistent with the Lubbock classing office data of the 2006-2007 crop which showed that 40.5% of the bales had a micronaire of 3.5 or below. The HVI strength of the TM-1 fibers was higher for field-grown samples than for the green-house samples. This could be due to the very principle of HVI bundle testing. HVI bundle strength results from a force measurement divided by the weight. Lower is the micronaire larger is the number of fibers in the bundle for the given weight. This will result in higher bundle strength. It does not mean that individual fiber strength is better.

The HVI average elongation of TM-1 is 7.2% in the green-house and 5.7% in the field. Average strength and elongation values of *im* are lower than TM-1 for both, green-house and field-grown cotton. There is in general, a weak correlation between fiber maturity and HVI elongation that is not seen in this set of data. This could mean that in addition to cellulose deficiency *im* fibers may exhibit a structural organization problem.

AFIS data analysis: The AFIS measures length by number, length by weight, maturity ratio, fineness, neps and trash. Neps are entangled fibers formed primarily by immature fibers. Neps may lead to the formation of white specks on the dyed fabric (Davidonis, 2003, Mangialardi, 1987). The *im* fibers were found to be very immature and extremely fine (meaning low weight per km of fibers), in the green-house as well as in the field-grown plants (Tables 2-a and 2-b). Green-house grown fibers have 6 times less neps compared to the field-grown *im* fibers. This indicates that the phenotype *im* is greatly affected by the environmental conditions. Field-grown *im* also has 7 to 8 times higher proportion of neps compared to field-grown TM-1. A high level of neps is very strongly suggestive of highly immature fibers. TM-1 fibers had better maturity in the green-house and much less neps.

Maturity distributions also show better maturity for TM-1 compared to the *im* plants in the field as well as the green-house (Figures 1-a and 2-a). Length distributions for the green-house and field-grown plants show a very high short fiber content for *im* compared to TM-1 (Figures 1-b and 2-b). The high short fiber content is probably resulting from fiber breakage (during ginning and/or in the AFIS opener) due to the immaturity of the fibers. It seems unlikely for the *im* plants to have a genetic predisposition to shorter fibers, since previous studies showed that *im* continues to grow in the elongation phase up to 21dpa (Kohel, 1974).

Variability of fiber quality within a plant

We investigated fiber quality on twelve branches, on boll #1. The lint collected was not sufficient for HVI testing and hence we performed only AFIS testing on these samples and plotted length and maturity distributions.

For the different positions in the TM-1 plant, we found decreasing maturity ratio from the bottom of the plant to the top of the plant as shown in Table 3-a. The number of neps per gram and the immature fiber content steadily increased from the bottom to the top of the plant while logically fiber fineness (mtex) decreased. The mean fiber length also decreases from the bottom branch to the top. For *im* plants, AFIS data showed a low maturity ratio for all the positions within the plant (Table 3-b). As expected, the nep count was very high in most of the immature samples, with all the samples being extremely fine and having a high immature fiber content. Length variation among bolls does not vary much for *im*.

Maturity and length distributions were plotted using the data from the AFIS. For TM-1, maturity ratio distribution shifts towards lower maturity from branch 1 to branch 12 plant (Figure 3-a). Fiber length distributions show a gradual increase in short fiber

content (Figure 3-b). It is reasonable to assume that decreasing maturity leads to weaker fibers that break easily, generating more short fibers.

For *im,* maturity ratio distribution shows very low maturity regardless of the branch location within the plant (Figure 4-a). The length distribution show very high short fiber contents, which tend to decrease at the top of the plant (Figure 4-b). It is noteworthy that for *im* fibers, bolls located at the top of the plant have lower short fiber content and better maturity ratios.

For the TM-1 field-grown plants, based on the average sample HVI micronaire, one would conclude that this cotton is of poor quality. However, based on AFIS results, we observe that not all bolls have poor quality. It is the production from the top of the plant that brings down the overall fiber quality. Therefore, in order to take a representative lint sample, sampling techniques are of utmost importance. For research purposes and when dealing with individual plants, it is very important to apply an appropriate sampling technique (with bolls from all parts of the plant) because of the tremendous variation observed within a plant. Figure 5 shows the overall evolution of the AFIS maturity ratio of cotton samples picked from branch 1 up to branch 12 for both TM-1 and *im*. For *im* fibers, there is no variation in maturity ratio within the plant. However, for TM-1 the maturity ratio varies from 0.92 for branch 1 position 1 down to 0.76 for branch 12, position 1. It is observed that TM-1 and *im* differ greatly, in their distribution of fiber quality within a plant.

The declining quality of fibers from the bottom of the plant to the top is probably due to the indeterminate growth of the cotton plant (Deterling and Kamal, 1982). The lower branches receive maximum nutrition compared to the top branches. Also, UV radiations weaken the fibers (Morton and Hearle, 1997) and the topmost branches are

exposed to direct sunlight a lot more than the bottom branches. We observe that at about branch number ten, for TM-1 plants, the fiber quality begins to be very similar to that of the *im* fibers (Figure 5).

The green-house grown cultivars have better quality compared to field-grown cultivars due to more controlled environment in the green-house. The *im* plants also show less severe phenotype in the green-house and looked healthier. The tight-lock boll pattern described by Kohel et al, was observed in both the green-house and field-grown plants (Kohel et al., 1974).

Based on the result of this preliminary study, we have suggested a sampling protocol for breeders and biotechnologists to avoid errors in fiber quality analysis contributed by fiber quality variability. This protocol involves harvesting all the bolls or one lock/boll of the entire plant followed by ginning. The sample can be saw/roller/hand ginned depending on the type of germplasm used or depending on the goal of the project. The lint obtained, is then blended. This mixed sample would thus, be representative of the overall fiber quality of the entire plant.

CONCLUSIONS

Fiber quality varies drastically depending on where the fibers are picked from within the plant. Analyzed fibers need to be representative of the plant or genotype tested. Therefore, an appropriate sampling protocol is very important for reliable fiber analysis. This is needed particularly when dealing with individual plants, or a small volume of fibers for analysis. Testing performed using HVI and AFIS suggested that for the TM-1 plants, the quality of the fibers declines from the bottom of the plant to the top. For *im*, the fiber quality among bolls was less variable.

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TABLE CAPTIONS

Table 1-a. HVI Data of green-house average samples.

Table 1-b. HVI Data of field-grown average samples.

Table 2-a. AFIS data of green-house average samples.

Table 2-b. AFIS data of field-grown average samples.

Table 3-a. AFIS data of TM-1 from branch #1 to branch #12.

Table 3-b. AFIS data of *im* from branch #1 to branch #12.

Table 1-a

ID	Rep	Micronaire Index	Length (in)	Uniformity Index (%)	Strength (g/tex)	Elongation (%)	Short Fiber Index(%)
im[(a)]	1	2.0	1.23	80.0	25.2	5.5	8.6
im	2	2.0	1.20	81.1	26.9	6.2	8.3
im	3	--no lint -----------------------------------					
Average		2.0	1.22	80.5	26.0	5.8	8.4
TM-1[(b)]	1	3.4	1.24	85.5	27.1	7.0	6.4
TM-1	2	3.0	1.22	84.1	27.5	6.9	7.0
TM-1	3	3.1	1.24	84.5	27.9	7.6	6.7
Average		3.2	1.23	84.9	27.5	7.2	6.7

(a) *im* immature mutant; (b) Texas Marker -1 wild type

Table 1-b

ID	Rep	Micronaire Index	Length (in)	Uniformity Index(%)	Strength (g/tex)	Elongation (%)	Short Fiber Index (%)
im [(a)]	1	2.0	1.07	76.7	22.5	3.8	13.3
im	2	2.0	1.03	75.6	20.5	5.5	15.4
im	3	2.0	1.02	76.0	19.9	6.1	15.2
Average		2.0	1.04	76.1	21.0	5.1	14.6
TM-1[(b)]	1	2.9	1.17	81.8	28.2	5.8	8.7
TM-1	2	2.6	1.16	82.0	30.5	5.8	9.1
TM-1	3	2.8	1.18	82.8	31.2	5.6	7.8
Average		2.8	1.17	82.2	30.0	5.7	8.5

(a) *im* immature mutant; (b) Texas Marker -1 wild type

Table 2-a

ID	Rep	Neps per g	Length (w) (in)	UQL[c] (w) (in)	Length (n) (in)	H[d] (mTex)	IFC [f] (%)	Maturity Ratio
im[a]	1	606	0.91	1.18	0.65	124	10.7	0.77
im	2	678	0.95	1.21	0.70	130	10.1	0.78
im	3	-------------------------------------			---no lint---			-------------------------------------
Average		642	0.93	1.19	0.67	127	10.4	0.77
TM-1[b]	1	101	1.08	1.29	0.91	167	6.6	0.86
TM-1	2	199	1.02	1.26	0.83	155	8.1	0.82
TM-1	3	139	1.05	1.29	0.84	157	8.2	0.83
Average		146	1.05	1.28	0.86	160	7.6	0.84

(a) *im* immature mutant; (b) Texas Marker -1 wild type (c) Upper Quartile Length

(d) Fineness (f) Immature Fiber Content

Table 2-b

ID	Rep	Neps per g	Length(w) (in)	UQL[c] (w) (in)	Length(n) (in)	H[d] (mTex)	IFC[f] (%)	Maturity Ratio
im[a]	1	2423	0.87	1.13	0.64	113	11.3	0.76
im	2	3620	0.78	1.02	0.55	112	13.8	0.72
im	3	3861	0.80	1.03	0.57	114	13.2	0.73
Average		3301	0.82	1.06	0.59	113	12.8	0.74
TM-1[b]	1	400	1.00	1.23	0.81	149	8.7	0.83
TM-1	2	565	0.96	1.22	0.75	141	9.3	0.81
TM-1	3	384	1.03	1.26	0.84	150	8.7	0.83
Average		450	1.00	1.24	0.80	147	8.9	0.82

(a) *im* immature mutant; (b) Texas Marker -1 wild type (c) Upper Quartile Length

(d) Fineness (f) Immature Fiber Content

Table 3-a

ID	Neps per g	Length(w) (in)	UQL(c) (w) (in)	Length(n) (n)	H(d) (mTex)	IFC(f) (%)	Maturity Ratio
TM 1-1	79	1.17	1.37	1.04	177	5.0	0.92
TM 2-1	63	1.19	1.36	1.07	180	4.8	0.92
TM 3-1	59	1.17	1.35	1.05	178	4.9	0.92
TM 4-1	76	1.16	1.34	1.03	174	5.3	0.91
TM 5-1	73	1.20	1.37	1.07	172	5.6	0.90
TM 6-1	94	1.14	1.34	0.98	156	7.0	0.87
TM 7-1	81	1.13	1.31	0.98	159	6.7	0.88
TM 8-1	134	1.07	1.28	0.90	157	7.3	0.87
TM 9-1	216	1.05	1.28	0.86	135	9.4	0.81
TM 10-1	248	0.99	1.24	0.76	130	10.3	0.79
TM 11-1	338	1.01	1.23	0.79	128	9.9	0.79
TM 12-1	642	0.89	1.09	0.70	128	11.2	0.77
Maximum	642	1.19	1.37	1.07	180	11.2	0.92
Minimum	59	0.89	1.09	0.70	128	4.8	0.77
Average	175	1.10	1.30	0.94	156	7.3	0.86

(c) Upper Quartile Length (d) Fineness (f) Immature Fiber Content

Table 3-b

Sample	Neps per g	Length(w) (in)	UQL[c] (w) (in)	Length(n) (in)	H[d] (mTex)	IFC[f] (%)	Maturity Ratio
***im* 1-1**	1138	0.90	1.20	0.62	116	11.4	0.75
***im* 2-1**	559	1.03	1.30	0.77	124	9.1	0.80
***im* 3-1**	1041	0.97	1.24	0.72	119	10.6	0.78
***im* 4-1**	1304	0.97	1.26	0.71	118	10.2	0.78
***im* 5-1**	1896	0.91	1.17	0.65	116	10.8	0.76
***im* 6-1**	2332	0.85	1.10	0.61	116	11.8	0.75
***im* 7-1**	2477	0.89	1.17	0.63	123	10.2	0.78
***im* 8-1**	2478	0.96	1.23	0.70	119	10.1	0.77
***im* 9-1**	744	0.95	1.21	0.71	118	11.1	0.76
***im* 10-1**	1274	0.98	1.22	0.77	125	9.1	0.80
***im* 11-1**	2692	0.96	1.23	0.72	111	10.8	0.76
***im* 12-1**	800	0.95	1.19	0.74	118	9.0	0.79
Maximum	2692	1.03	1.26	0.77	125	11.8	0.80
Minimum	559	0.81	1.10	0.61	111	9.0	0.75
Average	1561	0.94	1.21	0.70	119	10.3	0.77

(c) Upper Quartile Length (d) Fineness (f) Immature Fiber Content

FIGURE CAPTIONS

Figure 1-a. AFIS maturity ratio distributions of green-house average samples.

Figure 1-b. AFIS length (n) distributions of green-house average samples.

Figure 2-a. AFIS maturity ratio distributions of field-grown average samples.

Figure 2-b. AFIS length (n) distributions of field-grown average samples.

Figure 3-a. AFIS maturity ratio distribution of TM-1 field-grown samples.

Figure 3-b. AFIS length (n) distribution of TM-1 field-grown samples.

Figure 4-a. AFIS maturity ratio distribution of *im* field-grown samples.

Figure 4-b. AFIS length (n) distribution of *im* field-grown samples.

Figure 5. Comparison of maturity ratio at boll #1 at different branches of TM-1 and *im*.

Figure 1-a

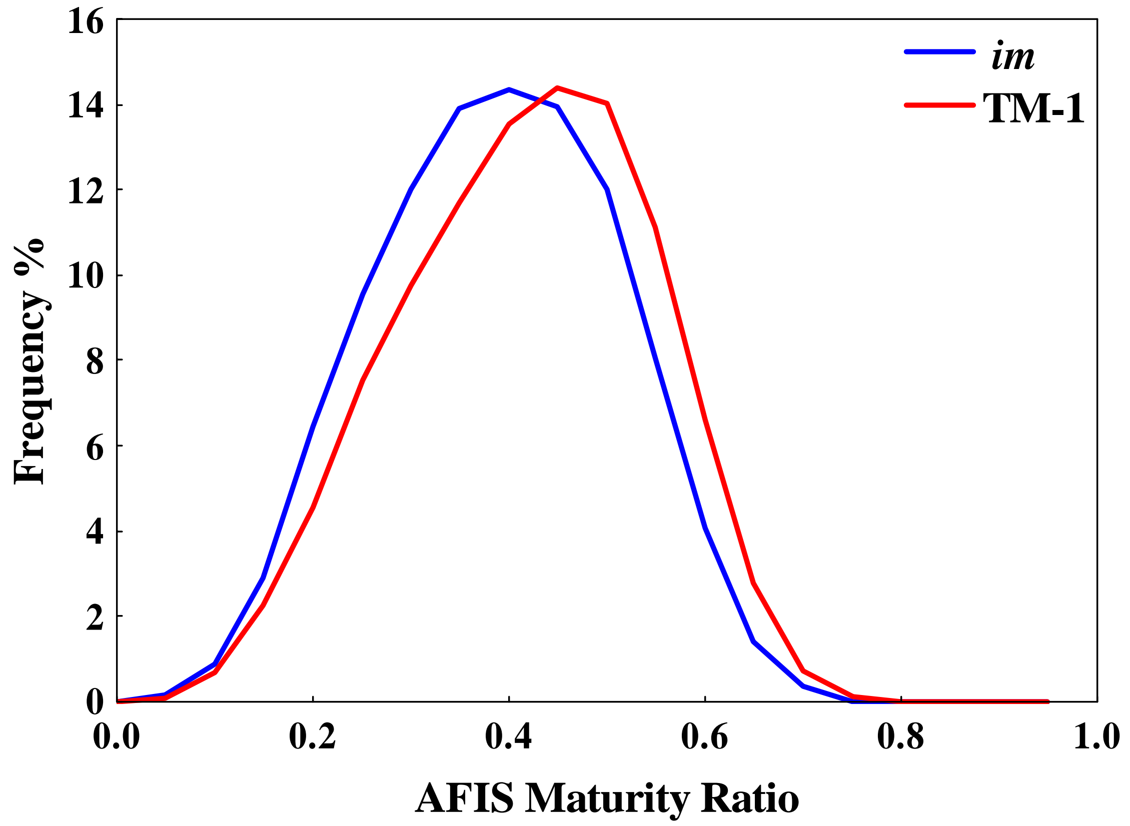

Figure 1-b

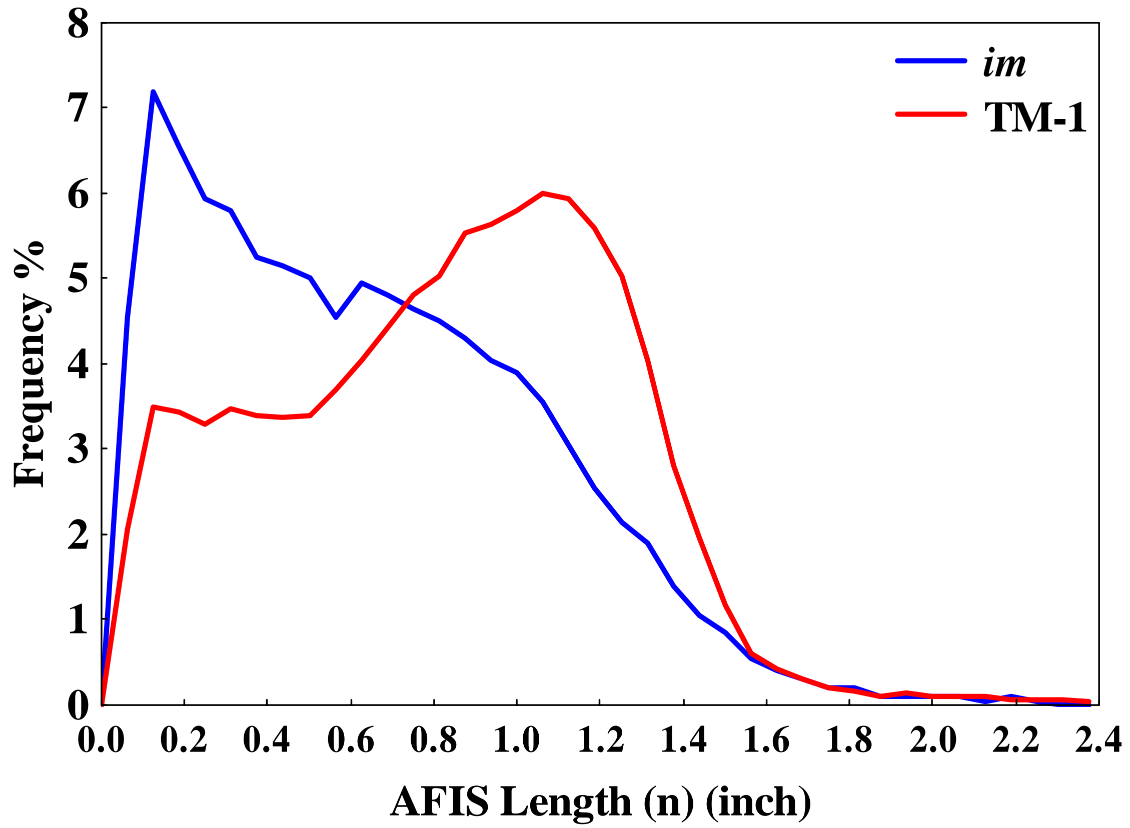

Figure 2-a

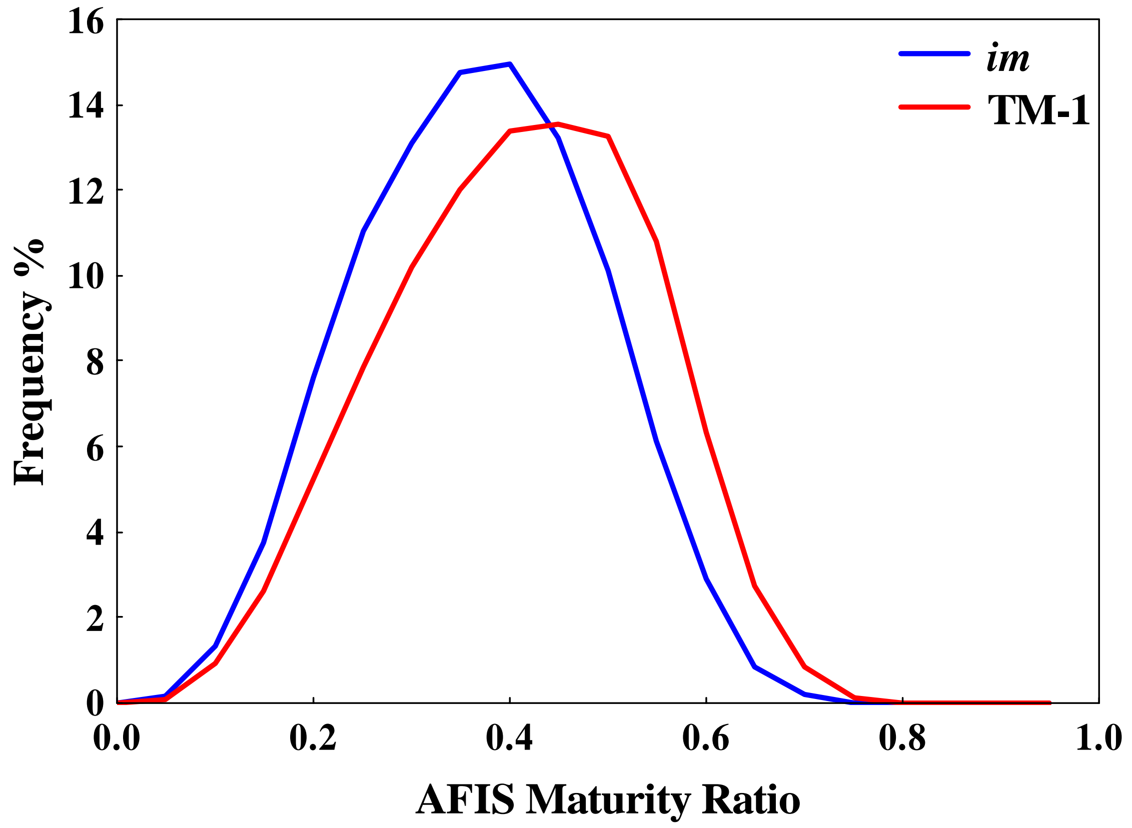

Figure 2-b

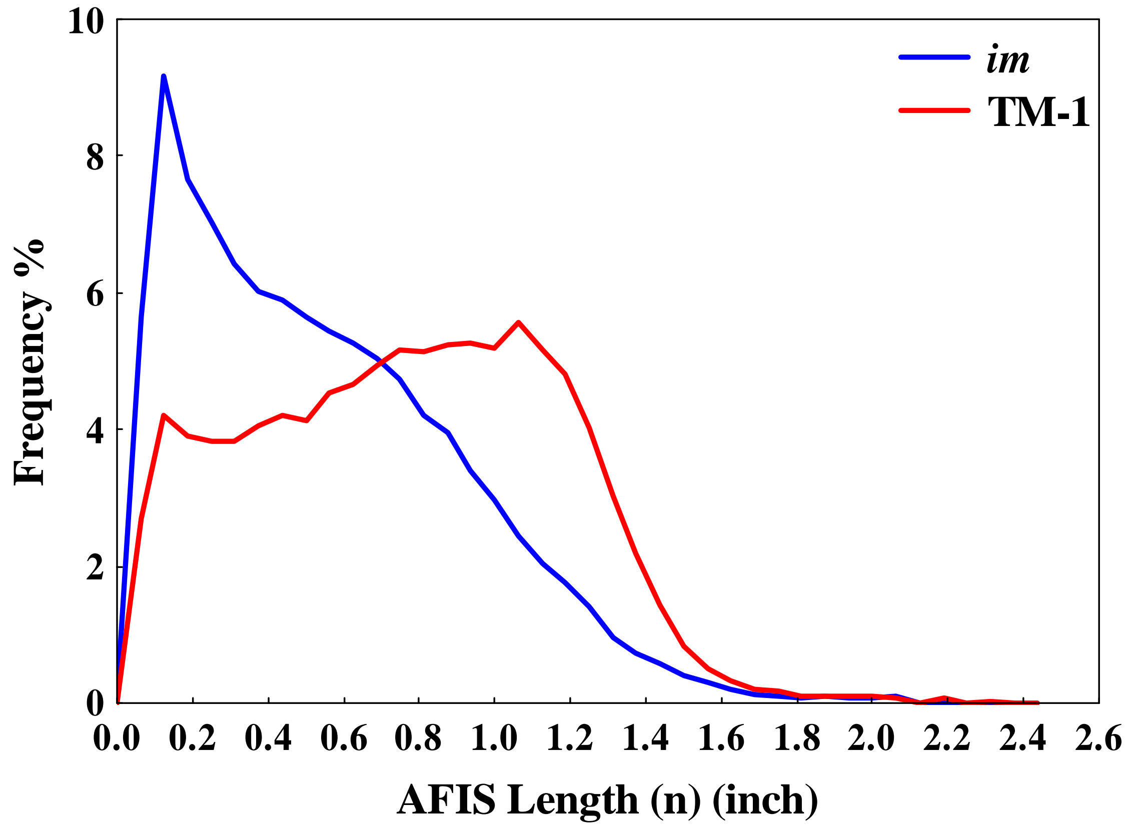

Figure 3-a

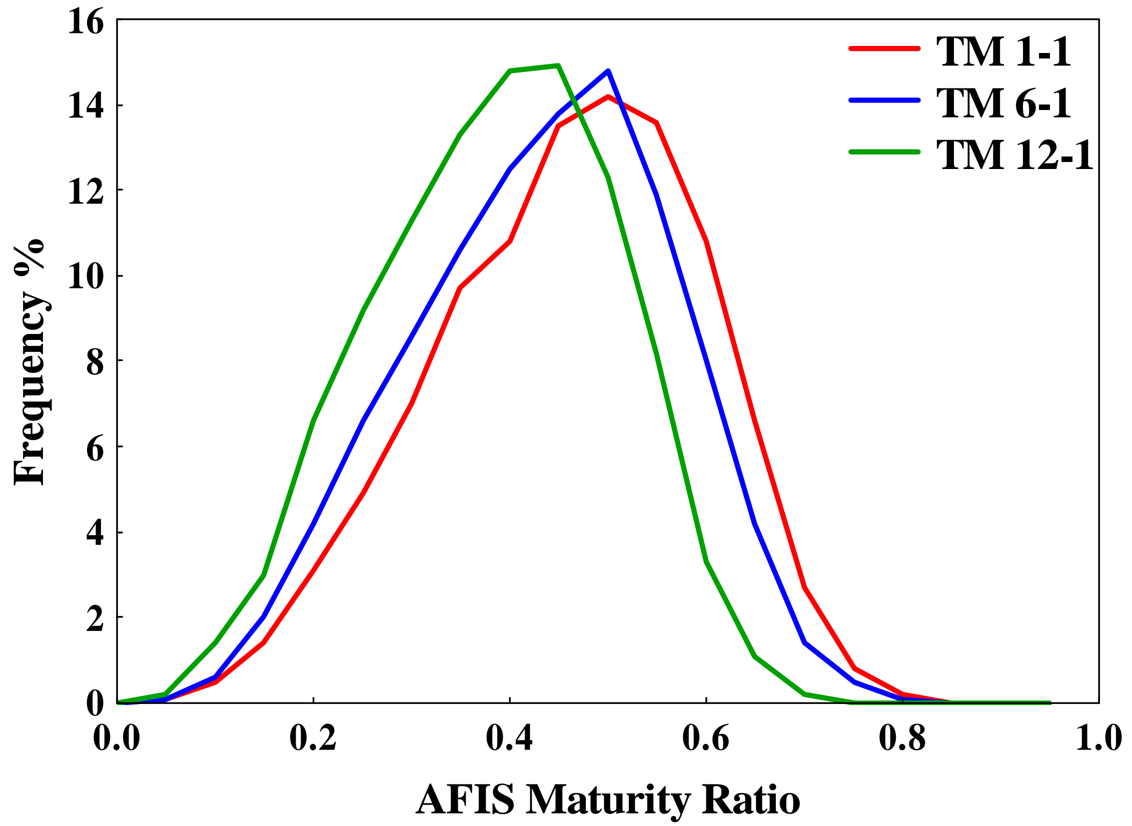

Figure 3-b

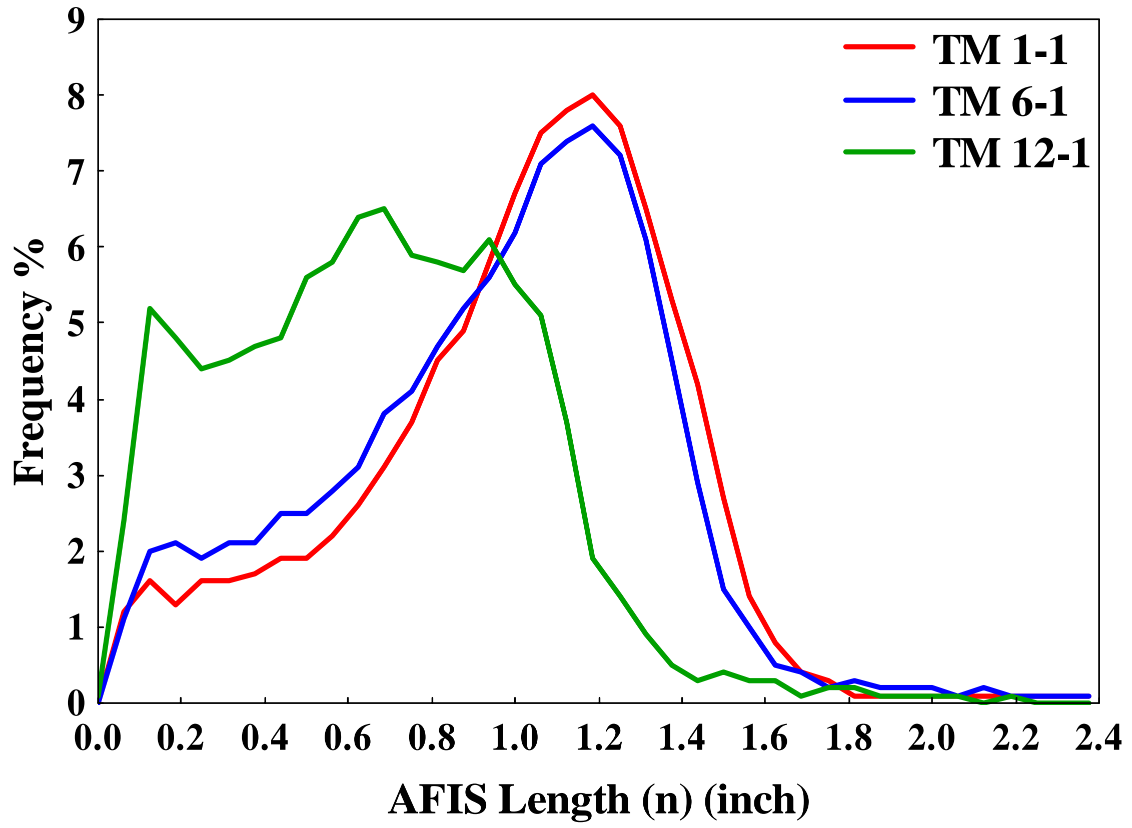

Figure 4-a

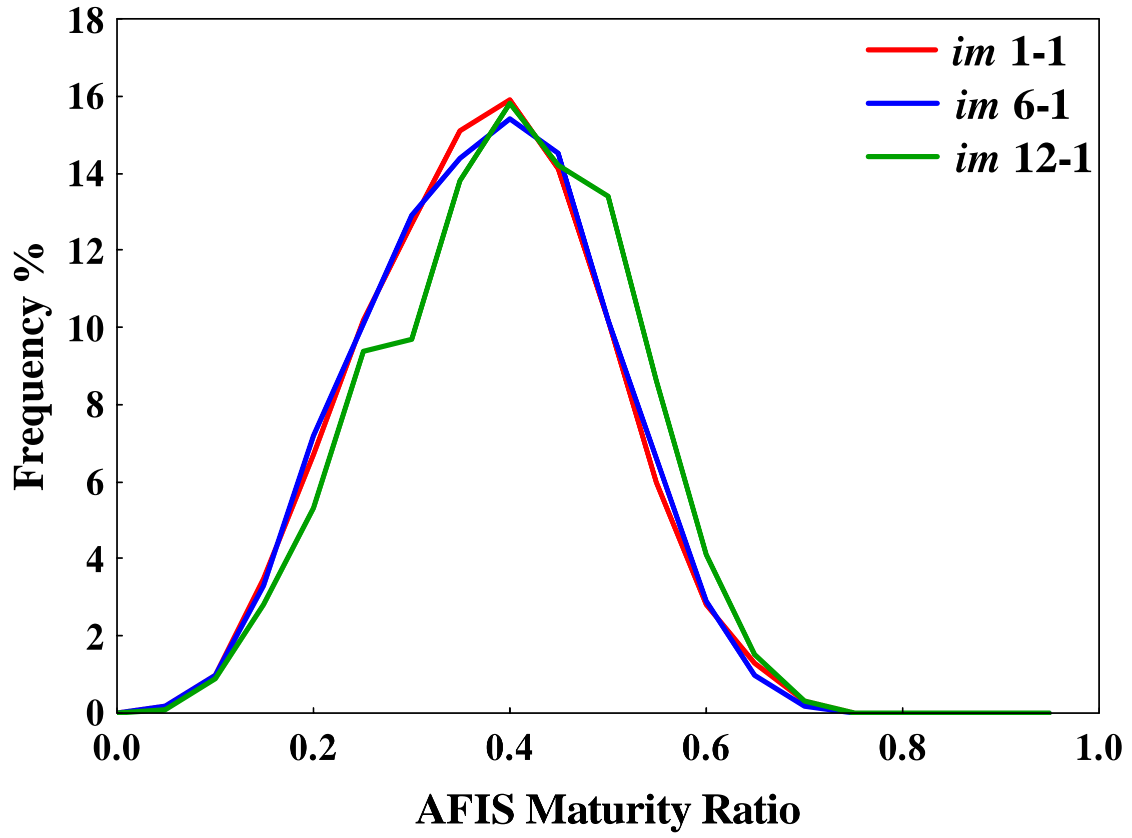

Figure 4-b

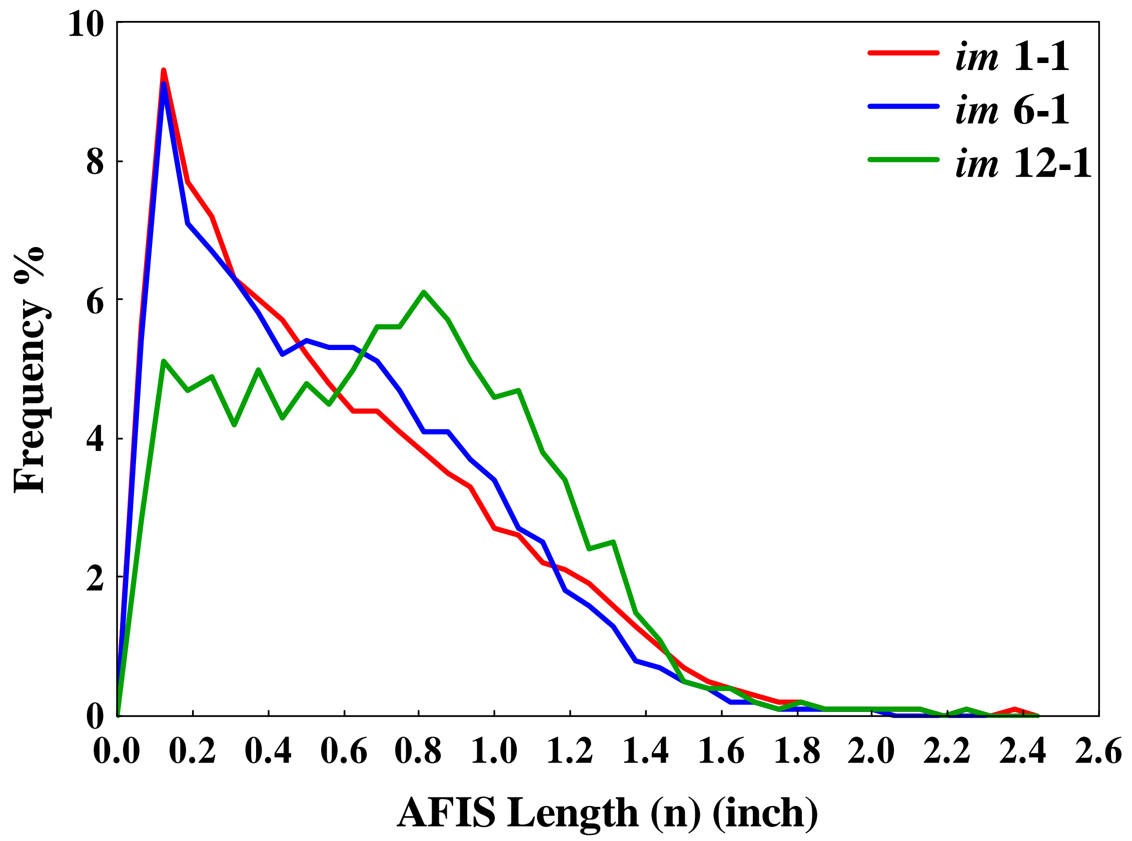

Figure 5