A Comparison of Morphological Maturity Methods in Upland Cotton for the Optimization of Crop Production and Breeding Systems

by

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Abstract

The indeterminacy of upland cotton (*Gossypium hirsutum* L.) necessitates methods for evaluating its reproductive status and progress. There is no universally accepted agronomic method for assessing cotton maturity and the method used in a breeding program needs to be quick and efficient within a limitted time frame. In this study, Node of First Fruiting Branch (NFFB), Node above White Flower (NAWF), Node above Cracked Boll (NACB), and visual estimation of percent open (Open) were collected on 16 elite upland cotton varieties at 11 total locations over three years. NAWF, NACB, and Open were each collected twice. Correlations were determined by using the Pearson correlation coefficient. Of all the agronomic maturity methods compared, the first and second measurements of NACB were the most highly correlated. Tukey's HSD was used to find significant differences between genotypes and genotype's maturity groupings. NFFB found the most statistical difference between individual genotypes but found no difference in the genotypes when grouped by maturity grouping. High correlation between the second Open and the first NAWF rating suggests Open can be a valuable substitute for the more time consuming NAWF and NACB ratings. This research indicates no one maturity method can fully assess the maturity of a genotype.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AGA	Trial location near Sasser, GA
ANOVA	Analysis of Variance
BGA	Trial location near Baxley, GA
CV	Coefficient of Variation
DAP	Days after planting
DF	Degrees of Freedom
H^2	Broad Sense Heritability
HGA	Trial location near Dawson, GA
HSD	Honest Significant Difference
HVI	High Volume Instrumentation; testing machine for measuring cotton fiber properties and quality
LGA	Trial location near Leary, GA
Loc	Location
MGA	Trial location in Mitchell County, GA
Mid	Middle Maturing Variety
MVGA	Trial location near Midville, GA
N	Number of Observations (plots)
NACB	Nodes above Cracked Boll
NACB1	The first measuring of Nodes above Cracked Boll in the season
NACB2	The second measuring of Nodes above Cracked Boll in the season
NAWF	Nodes above White Flower

NAWF1	The first measuring of Nodes above white flower in the season
NAWF2	The second measuring of Nodes above white flower in the season
NFFB	Node of First Fruiting Branch
Open	A visual estimation of percent open bolls
Open1	The first visual estimation of percent open bolls in the season
Open2	The second visual estimation of percent open bolls in the season
PGR	Plant Growth Regulator
Rep	Replication
SAS	Statistical Analysis Systems
SGA	Trial location near Sycamore, GA
SS	Sum of Squares
VGA	Trial location near Vienna, GA

CHAPTER ONE

Literature Review

Cotton Maturity

Cotton (*Gossypium hirsutum* L.) is a major textile fiber, as well as an important oilseed crop. The genus *Gossypium* (L.) has 50 species in total. *Gossypium hirsutum* L., also known as upland cotton, or cotton, is the most widely grown species. The fiber is used to spin into yarn, which is used to make clothing, towels, curtains, etc. (Stewart and Rossi, 2010). The seed contains a large amount of oil (16%-27%), which is extracted for use as a vegetable oil. Whole cottonseed and the pressed leftover seed from oil extraction are used as a feed additive for livestock (Dowd et al, 2010). In 2020, in the United States, 8.7 million acres of cotton were harvested, with an average lint yield of 825lbs/acre. This lint had a total value of 4.7 billion US dollars. The cottonseed produced in 2020 was valued at 903 million dollars (USDA NASS, 2021).

Upland cotton is a perennial shrub, usually 1 to 2 m tall. It is commonly grown as an annual. The leaves have three to five lobes, which are triangular to ovate in shape. The petals are up to 50 mm long. Upland cotton is indigenous to Middle America and the Antilles, as well as some Pacific Islands (Fryxell, 1984). Upland cotton is an indeterminate crop that produces two types of branches: vegetative branches (monopodium) and fruiting branches (sympodium). Sympodium can be differentiated from monopodium by looking at the terminating node on the branch. Monopodial branches produce leaves at successive nodes until stress causes growth to end. Sympodial branches terminate in a fruit form. Additionally, due to the axillary bud breaking to produce each new fruiting node, sympodium has a characteristic of zig zag pattern (Elsner, 1979). Monopodial branches develop from the main stem below sympodial branches but often do

not make a significant contribution to the overall yield. Both types of branches grow simultaneously once fruiting has begun (Whitaker et al, 2019). The arrangement of branches from the main stem is called phyllotaxy. Cotton has a spiral phyllotaxy, with each node being a 3/8 turn above the previous. This can be clockwise or counterclockwise (Mauney, 1984). As the cotton plant matures, it allocates carbohydrates to fruit production. At the same time, development of new mainstem nodes slows, so the first position white flowers appear progressively closer to the plant apex (Waddle, 1974).

Cotton squares, flowers, and bolls occur on a regular and predictable schedule. The accepted standard is three days between vertical nodes and six days between horizontal fruiting positions (McClelland, 1916). However, Bednarz and Nichols (2005) found the time intervals between successive node growth could be a little shorter. This development of the cotton plant is extremely predictable. Nonetheless, cotton plant growth and development are influenced by temperature, soil moisture, nutrient availability, and genotype (Gipson and Ray, 1970).

A fruiting position begins with a square, or bud. It has a distinct three-sided structure formed by the bracts, which enclose the bud. Inside these bracts are the developing parts of a perfect flower which contains both male and female sexual structures. The time required for the development of a small square (pinhead square) to transform into an open flower is 20 to 30 days under normal growing conditions. Most of this growth in size happens the week before bloom. Young squares are extremely sensitive to environmental stressors. Many young squares abort due to water, nutrient, insect, and other sources of stress. Even the “old adage [for cotton]” from 1851 holds true today, “time once lost can never be regained.” (Jefferson, 1851).

The cotton flower opens in the early to mid-morning due to the petals rapidly expanding in the previous 24 hours. During this time, the stigma also expands. The appearance of the first

flower indicates the plant has reached the reproductive stage. Shortly after the flower opens, pollen sheds from the anthers and fertilizes the pistol, and subsequently, the ovule. (Mauney and Stewart, 1986)

Cotton, with its perfect flower, is normally a self-pollinating crop. However, natural out-crossing may occur up to 50%. As cotton pollen is heavy and sticky, it is not conducive to transfer by wind. Insect pollinators are the primary method of cross pollination. Common insect pollinators are bees, specifically bumblebees (*Bombus* spp.) and honeybees (*Apis mellifera*), though other insects may contribute to the cross pollination. (Niles and Feaster 1984)

Cotton pollen is short-lived. Even in optimal conditions, pollen is viable for one day once the flower opens. If an ovule is not fertilized, it soon abscises. When the flower first opens, it is white. The next day, the flower turns pink to magenta to purple in color. Subsequently, the flower dries, and often falls off. The dry flower, if it remains attached to the developing boll, is often referred to as a bloom tag. If the ovary is fertilized, it continues to grow and develop, reaching its full size and weight 3 to 4 weeks after pollination. Concurrently, seed reach full volume. Developing fibers, that grow as hollow tubes from the surface of the seed coat, finish lengthening during this period. Although this species has germplasm with varying colors of fiber, commercially grown genotypes have been bred to have white or nearly white fibers. The boll is mature when it first opens, or “cracks.” At the time of cracking and immediately before this, the seed coat finishes maturing. The walls of the boll begin to dry and reflex open. The number of days it takes for the bolls to fully open are mainly determined by temperature and relative humidity. Finally, the boll has opened to its most recognizable form. (Mauney and Stewart, 1986)

Bolls require fewer carbohydrates the further they are out from the mainstem node, when compared to first node fruiting positions. The continuous production of fruiting positions and indeterminacy of the plant makes maturity difficult to assess. Cotton is affected by the sink-source ratio. This ratio is the relationship between vegetative and reproductive growth, root, and canopy growth, as well as the carbon and nitrogen balance. (Kong and Dong, 2011)

The growing period of cotton is dependent to the minimum yield accepted. Waddle (1984) states 120 to 200 growing days may be required to gain acceptable yields. A common planting window for the southeastern United States is April 1 through May 25, with early April planting uncommon. Little yield difference has been shown within this planting window. (Whitaker et al, 2019) Regardless of how long the cotton plant takes from planting to harvest, the timing of cotton from planting to first bloom is approximately the same. Cotton is suitable for growth in areas with a wide range of rainfall. Texas and Oklahoma may receive 50 cm (19.7in) of rainfall annually, while the Southeast normally receives 150 cm (59.1in) of rainfall per year. A water deficit can reduce yields. The rule of thumb is cotton grown west of 100 degrees west Longitude requires irrigation. In contrast, some coastal areas of the southeast may have excessive rainfall for optimal cotton production. Common rains in the late fall usually require farmers to harvest the crop before these rains begin. Worldwide, more than 60% of cotton is irrigated. (Waddle, 1984)

Cotton can be grown in a wide variety of soils successfully. In the United States, any soil south of the 37 degrees North Latitude can be suitable for cotton production. The 37^{th} parallel reaches over a curved area of the United States, starting west near Santa Cruz, CA, through the borders of Oklahoma and Kansas, and to the east stops in the US in the Chesapeake Bay in Virginia. The areas south of this latitude which are not conducive to cotton production are

usually prone to flooding. Cotton's most desired soil, when considering moisture retention, are the silt loams. Cotton grown in sand or clay may require extra maintenance. Additional irrigation may be necessary for a sandy soil. Clay soils are prone to water saturation, and subsequently oxygen loss. Cotton roots stop working when the oxygen levels in the soil fall below 10% (Huck, 1970). Even though cotton can be grown on almost any soil south of the 37th parallel, the farmer must decide if it is economically feasible to do so, considering the extra maintenance of the crop due to the soil type, in conjunction with input costs and market conditions that effect the net return per acre for the producer. (Waddle, 1984)

Prior to planting, many farmers collect soil samples to test for pH and the nutrient levels present. Then, with knowledge of their current levels and the anticipated yield, the needed mineral nutrients, in the form of fertilizers or lime can be calculated. The nutrients cotton requires the most of are nitrogen, phosphorus, and potassium. Calcium and magnesium are also important, but in lesser quantities. Calcium plays an important part of plant senescence. A deficiency in magnesium can limit fruit production. (Benedict, 1984) Calcium and magnesium usually already reside in the soil in adequate amounts (Waddle, 1984). Senescence is a normal process in cotton. It is a part of the normal maturity, which helps utilize limited energy and resources in the growing season. However, premature senescence will reduce yield and fiber quality (Dong et al, 2006). Premature senescence can be caused by drought during boll setting and opening. This water shortage will lead to a reduction in photosynthesis and shorten the growing period. (Chastain et al, 2014)

Nitrogen is needed in the highest quantity for fruit production; a shortage of the nutrient affects plant growth and maturity. The rate of nitrogen application also has a significant effect on cutout date, overall plant height, and total number of mainstem nodes. When the cotton plant has

insufficient nitrogen for plant growth, it will hasten its maturity and reach cutout sooner. (Snider et al., 2021) Cutout is a common term for describing a moment in a cotton crop's maturity. It is the point in the lifecycle of the cotton plant where cessation of new vegetative growth occurs. (Snider et al., 2021) Although nitrogen has been found to affect cotton growth rates, leaf area, boll shedding, yield, and response to water stress, it does not have an affect on time to first flower, time to first open boll, flowering interval, and number of seed per boll (Radin and Mauney, 1982)

Phosphorus promotes the cotton plant to transform from vegetative to reproductive growth. It also plays a role in seed maturity, boll weight, and can accelerate boll opening in the late growth stage. Phosphorus deficiency causes chlorosis, slow growth, reduced yield, and delayed maturity. (Rochester, 2010)

Potassium availability has a great deal to do with cotton maturity. Potassium deficiency reduces chlorophyll content in leaves, root activity, and leads to premature senescence. (Dong et al, 2004) Deficiency in this nutrient also decreases the sink from the leaves to the bolls, leading to excess energy being transferred to other sinks (Pettigrew, 1999, Lim et al, 2007). Cotton's natural salt tolerance can be beneficial, as the crop can be grown on land not suitable for other crops. However, if salt levels are too high, salt ions will replace the potassium ions, causing a potassium deficiency. (Wang et al, 2014) High salinity levels are most harmful to young seedings. Field flooding to move the salts to lower levels of soil or the use of cover crops can reduce the soil's salinity to tolerable levels. (Waddle, 1984) Boron is commonly added as a foliar spray 45-60 DAP (days after planting), as this nutrient is essential for fruit setting and retention. (Waddle, 1984) More specifically, Boron influences the translocation of carbohydrates. A

deficiency in boron is characterized by short sympodia and young bolls failing to develop. (Benedict, 1984)

Biologically, hormones within the plant control its reproductive process. Gibberellic acid promotes cell growth and elongation (Ray, 1997). Anthesins induce flower formation. There are many more hormones which effect flowering in cotton. The ratios of these hormones dictate the growth and reproduction of the cotton plant. (Benedict, 1984) Several genes (GA2OX2, GA2OX6, and GA2OX8) have been found to inactivate gibberellic acid, which affects flowering period. (Rieu et al., 2008; Schomburg et al., 2003). Too much gibberellic acid may delay leaf senescence (Yu et al, 2009).

Growers commonly spray plant growth regulators (PGRs) to control shoot growth. PGRs promote the retention of existing bolls, which helps yield. When applied with the correct rate and timings, PGRs aid fiber quality by diverting nutrients to the fruit set during peak bloom. (Kerr and Royster, 1977) A common PGR is mepiquat chloride, which limits vegetative growth by redirecting the carbohydrates towards the plant's reproductive growth. PGRs are also utilized to reduce the height of the plant, which is useful for mechanical harvest. (Ray, 1997)

Yield is maximized partly by choosing the most suitable upland cotton variety, or genotype for the area. Cotton yield is primarily contingent on the number of bolls set per acre, the weight of each boll, and the proportion of weight the lint contributes to the overall boll weight, or lint percent. Before 1900, cotton cultivars were mainly large and slow-fruiting plants. They were full and long season varieties. However, after the introduction of the boll weevil (*Anthonomous grandis* Boh.), preference was given to cultivars that bloomed more quickly, and earlier in the season. (Hintz and Green, 1954) Bollworms and 'catterpillers' would descend on cotton crops in Alabama around 1 Sept., destroying all bolls that were not yet firm. An earlier

maturing genotype could prevent this yield loss (Jefferson, 1851). Even after the eradication of the boll weevil, earliness, or the maturity, in upland cotton has remained of great importance. As mechanical harvest became common, plant structure and maturity were prioritized differently. The maturity of the genotype being consistent across the different plants in the field is important, so the harvester only runs through the field once. A uniformly maturing genotype not only helps with efficient harvesting, but also better fiber quality. (Colwick et al., 1984)

Yield can also be reduced due to weed pressure. The growth stage of the crop when weed competition occurs is critical. The first two months after planting is when cotton is most susceptible to weed pressure. This can depend on the maximum period weeds can be tolerated without affecting yield, and the period after weed growth does not affect final yield. (Ridgway et. al, 1984) Weeds in cotton do more than just reduce yield. Weed pressure can also reduce the quality of the cotton as well as increased costs for weed removal through herbicides, tillage, or by hand. Weeds in a cotton field can prevent proper water management as well as compete with the cotton for limited resources, like nutrients and sunlight. The weeds themselves can provide habitats for nematodes, insects, disease, and rodents. Other than in indirect ways, weeds do not affect the maturity of cotton. (Shaw, 1964)

Partly because of cotton's indeterminacy, in the US, cotton is terminated chemically. Applying the termination chemicals, or defoliant at the proper crop stage is imperative. Premature defoliation can increase short fiber content and neps. Likewise, harvesting the crop prior to 60% open boll can cause increased neps and reduced micronaire. (Williams and Bange, 2019) Micronaire outside of a standard range – either too high or too low – and increased short fiber content all are undesirable (Bange et al., 2010). High micronaire has a negative impact on dyeability of yarn and fabric, while a low micronaire creates reduced efficiency of yarn spinning

(Bradow et al, 1996). Although producers are not often penalized monetarily for a high presence of neps, a reputation can be gained for the source having low quality at the spinning mills (Gordon et al., 2004). To prevent or at least minimize these issues, it is important to understand crop maturity to determine the selection and timing of harvest aid products as well as the timing of harvest. A delay in defoliation or harvest subjects the crop to weathering and other factors that potentially reduce yield and fiber quality.

Maturity matters to the individual grower not only in genotype selection, but also because open cotton bolls tend to lose weight and quality each day they are open. Delaying harvest for a single week can reduce yield by 8%, and overall financial returns by 9%. A delay of harvest for two weeks can reduce yield and economic return by 23% and 25%, respectively. Compounding this is the fewer number of hours of daylight per day as the harvest season progresses. This shorter daylight time contributes to reduced efficiencies of harvesting. A shorter production season can be beneficial in increasing yield and efficiency, which can lead to larger profits (Parvin et al, 1987).

Quantification of Cotton Maturity

The term “maturity” is used to describe two slightly different but related concepts: the progress of the plant towards harvest (proportion of nodes with mature, harvestable fruit) and the length of growing season required by a particular variety (total number of nodes produced). (Mauney and Stewart, 1986) Lee (1987) defined earliness [maturity] as a measure of the time required for a genotype to produce a satisfactory crop under prevailing conditions. Bourland et al. (1992) defined cotton crop maturity by the date physiological cutout occurs. In the southeastern United States, Bednarz and Nichols (2005) found a node above white flower (NAWF) of 3 to be a reliable indicator of cutout.

Niles and Feaster (1984, p. 222) stated:

> Properly, 'earliness' should be viewed in respect to its components, which include seed germination and stand establishment, onset of squaring, onset of blooming, rate of blooming, boll retention, boll maturation period, and time of crop maturity.

As there is not one common accepted definition of maturity, it is logical there is not one accepted method for determining it.

Quantifying the maturity of a cotton genotype is important both for the breeders producing the varieties and the farmers growing them. Breeders target varieties to different regions in part based on crop maturity. Farmers often select varieties to plant partly on the reported maturity. Typically, early maturing cultivars are planted in regions where the climate limits the effective length of the growing season or in situations in which a crop is planted late in the normal period of crop establishment, such as in double cropping. Double cropping cotton is feasible in the southern region of Georgia with early maturing varieties under intensive management. (Whitaker et al., 2019) It is important in double cropping to choose a shorter season (earlier) genotype. Smith and Varvil (1982) found double cropped short season genotypes yielded 35 to 50% less than those when monocropped. They also found the double cropped full season genotypes yielded 50 to 65% less than monocropped. Therefore, short season genotypes lose less potential yield in each cycle when double cropped, when compared to full season genotypes. In the western United States, safflower is a common winter crop in conjunction with cotton. More commonly, in the southeastern United States, cotton can be planted after winter wheat has been harvested. (Waddle, 1984) Full season or long maturing genotypes are more commonly accepted in the southern regions of the U.S. cotton belt. Though not always true, full season genotypes are often thought to be more stress tolerant or resilient in the presence of

temporary drought or other stresses. Constable and Bange (2015) suggest a full maturing and more indeterminate growing genotype (with also slow crop setting and a high number of harvestable bolls) could be part of unlocking the highest theoretical yield for cotton.

Earlier developing genotypes have been valued for their production efficiencies by reducing inputs, such as fertilizer, water, energy, and crop protectants (Niles and Feaster, 1984). Rapid fruiting genotypes can be beneficial in producing adequate yields in a short period of time, which provides the opportunity to grow a profitable crop and avoid the late season attack of pests, as well as make full use of all the heat units in the growing season (Ridgway et al., 1984).

The agronomic maturity of cotton can be determined using a variety of methods (Richmond and Radwan, 1962). Knowledge of the cotton crop's maturity assists in production management, including the timing of pest management inputs, PGRs, irrigation, and harvest aids (Gwathmey et al, 2016). Historically, cotton maturity was determined by measuring the percent of the eventual total picked weight that was picked at the first of two harvest dates. The use of boll-opening harvest aid products such as ethephon and the high costs of mechanical harvest eliminated the common practice of multiple harvest times and converted cotton systems and research to once-over harvest (Schaefer et al, 2016). The timing of harvest aid application and subsequent harvest has become a key decision point of modern cotton production. Modern maturity methods focus on either the node of the first position fruiting branch structure or the number of nodes remaining at the top of the plant as development slows. Due to the plant's predictable nature, a grower can choose a genotype and cultural practices so that the planting period and subsequent flowering and harvest periods coincide with a favorable local climate period (Waddle, 1984).

The quantification of cotton maturity is complicated by the lack of a standard unified method for doing so. Schaefer et al. (2016) suggests that more than one method needs to be used to accurately quantify the maturity of a cultivar.

Methods of Measuring Cotton Maturity

NAWF and Nodes above Cracked Boll (NACB) are historical methods of tracking cotton maturity (Schaefer et al, 2016). Nodes above cracked boll is useful in timing defoliation. If the crop is uniform and fruit retention is normal, Supak et. al (1993) supported the use of NACB as a means of timing defoliation. Gwathmey et al. (2016) found NAWF and NACB are useful for tracking cotton crop progress through the season. However, it should be noted, that Gwathmey et al. (2016) defines NACB differently than the research of Schaefer et al. (2016). Gwathmey et al. (2016) stops the count at the uppermost harvestable boll. Schaefer et al. stops the count at the apex of the plant. This research chose the Schaefer et al. (2016) method because the uppermost harvestable boll is somewhat subjective, as well as to maintain consistency with the NAWF count regarding the uppermost terminal as the end point.

NAWF was first described as a cotton maturity indicator by Waddle in 1974. It is used primarily as an in-season management tool (Bourland et al., 2001). NAWF is an earliness indicator, a general growth gauge, and can be collected by unskilled labor accurately and easily. Additionally, NAWF is a superior pre-harvest measure of earliness of maturity among genetically variable progenies (Waddle 1974). Bourland et al. (1992) found NAWF could assist in making end of season management decisions and defined cotton crop maturity by the date physiological cutout occurs, by measuring NAWF multiple times in the season and numerically expressing the maturity of a cultivar as days after planting (DAP) to NAWF = 5. Cotton plants reach first bloom generally from 55 to 70 DAP. Peak bloom is normally reached from 85 to 95

DAP. “Cutout” is a commonly used term that loosely indicates the near cessation of meaningful fruit production. Many accept the concept that cutout is reached when NAWF = 5 (Whitaker et al, 2019).

In breeding environments, a visual estimate of percent open bolls (Open) is often collected when the check lines are 50 percent open or when the variance between the lines is the greatest. The Open measure could be found by counting the open and not opened bolls, then dividing the opened bolls by the total number of bolls and multiplying by 100 (Whitaker et al, 2019). However, such counts are very time consuming. Even if done for a few row-feet per plot, it is still very demanding, and may not accurately represent the whole plot. With a visual estimation of Open, a breeder can look over the whole plot and then quickly assign a rating. Calhoun and Bowman (1999) states that a person experienced in performing this rating can evaluate 100 plots per hour. This is a practical method for a breeder who has thousands, or tens of thousands of plots to evaluate and doesn’t have time to count bolls.

End-of-season maturity (Open and NACB) measured data can be skewed by environmental factors that cause bolls to open prematurely (ex. Verticillium wilt), or prevent boll opening (ex. cold temperatures) (Bourland et al., 2001). Given the potential challenges of end-of-season issues, a mid-season maturity assessment may be more beneficial and accurate. For this reason, NAWF may be a better measure to use for distinguishing genotypes, while Open and NACB are useful for timing of harvest aid applications.

The most important factor when using Open to differentiate among genotypes is the timing of the rating. Taking the rating too early can cause too many leaves to obscure bolls. If the assessment is too late, all the plots may have most of their bolls open, masking any maturity differences. Experience is also key. The rater needs to be able to distinguish among numerous

open bolls and a smaller number of showy (loose) bolls (Calhoun and Bowman, 1999). To gain the most information from Open, the disparity among the genotypes open bolls percentages should be at its highest. If this is not achievable, then Open can be collected when the check with a known maturity is 50% open.

Node of first fruiting branch (NFFB) is a maturity method that does not change on a plant once it is established. Ray and Richmond (1996) found NFFB to be the most reliable morphological maturity measure as compared to number of vegetative branches and percentage of bolls on vegetative branches. Fan et al. (2004) found NFFB to be reliable for selection of early maturing varieties. Additionally, NFFB is a reliable maturity morphological measure and has high direct effects on the yield percentage before frost (Yu and Huang, 1990). Obviously, different methods have its own advantage and weakness, so the goals of this research are to compare the different agronomic maturity methods across different varieties; to determine which maturity method is a predictor of yield; to determine which maturity method is an accountable maturity method for breeding or testing programs; and to determine which method could differentiate between genotypes, or maturity groups of genotypes.

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CHAPTER TWO

Comparing Cotton Maturity methods for efficiency and characterization of cultivars in Upland cotton

Abstract

Quantifying a single genotype's maturity is a difficult task due to the influence of the environment, cotton's indeterminate nature, and the lack of a consensus among agronomists. In this study, Node of First Fruiting Branch (NFFB), Node above White Flower (NAWF), Node above Cracked Boll (NACB), and visual estimation of percent open (Open) were collected on 16 elite upland cotton varieties at 11 total locations over three years. NAWF, NACB, and Open were each collected twice. Correlations were determined among the maturity methods. Of all the agronomic maturity methods compared, the first and second measurements of NACB were the most highly correlated to each other. The second most correlated was a negative relationship between the first NAWF measurement and the second percent open visual estimation. NFFB was highly heritable but not correlated with any of the other maturity methods. The high correlation between Open2 and the first NAWF rating suggests Open can be a valuable substitute for the more time consuming NAWF and NACB ratings. However, this research found no one maturity method can fully assess the maturity of a genotype.

Introduction

Maturity has been recognized as an important trait of crops, particularly cotton, for a long time. Bennett (1908) deemed earliness [maturity] an important trait, stressing the setting of early bolls to attempt to avoid boll weevil damage. Despite the eradication of the boll weevil, maturity has remained an important trait.

Proper commercial varietal selection is essential to cotton growers. Yield potential is a major deciding factor, as are fiber quality, disease resistance, herbicide resistance, gene (pest management) trait package, maturity, and other factors. Choosing a variety with the proper maturity is imperative for each growing system. Knowledge of crop maturity is helpful for in-season decisions related to plant growth regulator (PGR) use, fertilizer applications, irrigation, and crop termination. Improper application of any of these could significantly reduce the yield and or the quality of the crop as well as the profit for the producer. Other benefits of earlier varieties include more time for fall land preparation and reduction in weather risks for harvesting (Bridge and McDonald, 1987). Smith (1984) found earlier varieties were beneficial for

- Avoid seedling diseases
- Escape from insect pests
- Avoid moisture related harvest issues
- Improve harvest efficiency
- Improve profitability with the possibility of double cropping

Contrary to this, full season varieties also have their benefits. They take advantage of the whole growing season to set bolls (Bridge and McDonald, 1987). Full season varieties can recover from episodes of stress earlier in the season and still produce a quality yield (Webb, 1984).

Seed companies categorize their varieties usually by early, mid (middle maturity), or full season maturity. Early varieties require the shortest growing season, while full varieties need a longer growing season but can take advantage of one as well. Some varieties can be in-between two categories. These could be considered early-mid or mid-full. To categorize the varieties, seed companies use a compendium of measures, usually collected over several years. Companies market varieties to different regions largely based on maturity. Marketing is another factor in maturity reporting. Misunderstanding cultivar maturity could lead a company to market an otherwise strong product in the wrong region, resulting in poor results, poor sales, and missed opportunities.

Objectives

The objectives of this research were to (1) compare the different agronomic maturity methods across different varieties to see if any methods are statistically the same; (2) determine which, if any, maturity method is a predictor of yield; (3) determine which, if any, maturity method is an accountable maturity method for assessing large numbers of populations in breeding or testing programs; and (4) determine which of the tested parameters could differentiate between genotypes, or maturity groups of genotypes.

Materials & Methods

A three-year study was conducted 2017-2019 across the cotton belt in Georgia to determine the best method of correlating morphological maturity methods for use in a high through-put breeding program. The trial consisted of 14 commercial varieties and 2 advanced BASF test cultivars with a wide range of maturities. They were planted in two row plots in a randomized complete block design. The cultivars were: BASF-9, BASF-10, CG3885B2XF,

DG3385B2XF, DP1252B2F, DP1538B2XF, DP1646B2XF, FM954GLT, NG5007B2XF, PHY333WRF, PHY444WRF, ST4747GLB2, ST4946GLB2, ST4949GLT, ST5517GLTP, and ST6182GLT. CG3885B2XF and DG3385B2XF were not grown in 2019 due to a lack of seed available. In 2017, the locations planted were Mitchell county, GA (MGA), Sasser, GA (AGA), Vienna, GA (VGA), and Midville, GA (MVGA). The locations planted in 2018 were Leary, GA (LGA), VGA, MGA, Sycamore, GA (SGA), and Baxley, GA (BGA). The locations in 2019 were LGA, VGA, BGA, and Dawson, GA (HGA). All plots were grown in fields rented from commercial cooperators, except 2019 HGA, which was located on the BASF cotton experimental breeding station near Dawson, GA. Row width and soil types at each location are shown in Table 1. All fields were under center pivot irrigation. Crop protection, PGRs, and irrigation were determined by the cooperator and/or their consultant according to what was appropriate for local production according to the University of Georgia Cooperative Extension recommendations. Node of First Fruiting branch (NFFB), two ratings of nodes above White Flower (NAWF1, NAWF2), two ratings of Nodes above Cracked Boll (NACB1, NACB2), two visual estimations of percent open bolls (Open1, Open2), and yield data were taken for each cultivar. Data were collected in 2017, 2018, and 2019, but most harvest results were lost in 2018 due to Hurricane Michael's destruction of the cotton across trial sites.

Planting dates were within the normal window for the South Georgia region. Successful stands were achieved at each location and were aided by timely irrigation. All locations were planted at a rate of 2.5 seed per foot and had 30ft plots, except 2019 HGA, which was a 15ft plot, but the alleys were mowed two weeks before harvest, trimming the plot lengths down to 12 feet. No alleys were mowed in the other trials.

For each of the morphological maturity methods (other than Open), data were collected from 10 representative random plants from each plot, avoiding any end plants, with an average generated for each plot. Each replication's data were collected within the same day. Yield was extrapolated to lint pounds per acre from seedcotton weight per plot, lint turnout (percent), and the plot size. Plot harvest dates (Table 1) were determined by crop readiness and weather conditions.

Node above White Flower counts were collected two times in the season, starting approximately 60 days after planting (DAP) and then again approximately 14 days later. The second rating was collected at 74 DAP as plants began the 3rd week of bloom, the time Waddle (1974) stated was best for NAWF counts. Node above white flower was collected by finding a first position white flower, then counting upwards the number of nodes to the terminal. The node of the branch with the first position white flower equaled zero. The terminal node was the top true leaf one inch in size or smaller.

NFFB was collected approximately 60 DAP concurrent with NAWF counts. The date of this collection is not imperative as the NFFB does not change in a plant once it has been established. Starting with the node above the cotyledon scars as node one, each node above was counted until the first sympodium was reached. The presence or absence of fruit on this branch was not considered. As this measure does not change, NFFB only needed to be collected once per year per plot. Commonly, the first sympodium occurs at node 6 or 7 but can range from nodes 4 to 11 (Elsner et al, 1979).

NACB can be found by finding the uppermost first position cracked boll (to be counted as zero) and counting upwards to the terminal node of the main stem. (Schaefer et al, 2016) This morphological maturity method was first collected approximately 115 DAP, the expected DAP

for the first open boll, and then again at around 125 DAP. Other researchers use NACB in relation to the uppermost harvestable boll (Gwathmey et al, 2016). Since the uppermost harvestable boll is subject to variability by opinion, the method chosen for this research involved NACB to terminal node to provide more exact, quantifiable data.

The Open method is acquired by a visual inspection and was conducted at 125 DAP and again at approximately 135 DAP. The visual data represent an estimation rather than an actual count. Looseness and shape of the bolls were disregarded as factors. These aspects can influence the rater and must be actively ignored. Ideally in practice, this rating is taken when the largest amount of disparity of percent open exists in the field, so the rater may capture the difference among the cultivars.

All locations were harvested with a 4row Case 420CPX plot picker. A small seedcotton grab sample, approximately 400 grams, was collected from each plot for ginning purposes, except for 2018LGA, 2018VGA, and 2018MGA, where yield was lost due to a hurricane. The samples were ginned on a small research gin without a lint cleaner to determine lint percent. A 30 gram lint subsample was sent for fiber analysis to the internal BASF HVI (high volume instrumentation) lab in Mississippi. Seedcotton weights were recorded for each harvested plot and added to the grab sample seedcotton weights for the total seedcotton weight per plot. Then, lint plot weight was calculated by multiplying total seedcotton weights by lint percent. Yield was calculated by dividing the lint plot weight by the percentage of an acre each plot occupied, determining the lint yield per acre. A means comparison was completed by Pearson correlation coefficient. A type III (analysis of variance) ANOVA using SAS 9.4 (Statistical Analysis Systems) was run to find interactions between replication (rep), genotype, year, location, genotype by year, genotype by location, as well as to find if there were any significant

differences among each of the maturity methods, both among each other and yield. The test also provided the coefficient of variation (CV) for each maturity method over the years. From the data the ANOVA provided, broad sense heritability estimations were calculated for all methods studied. Broad-sense heritability was calculated as:

$$H^2 = \frac{V_G}{V_G + V_E}$$

Where V_G is the genotypic variance and V_E is the environmental variance. Means comparisons were generated using Tukey's Honest Significant Difference (HSD) test with P=0.05.

Results

The ranges and means of the maturity measured by different methods are similar to those previously published (Table 2). Of all the maturity methods, NFFB had the lowest CV indicating repeatability. NACB1 and NFFB had the highest H^2 among the maturity measures. NAWF2, NACB2, and Open2's heritability's were all relatively the same. Yield had the lowest heritability. Open1's H^2 was not able to be calculated. Open1 had the highest CV indicating that there are large variations among replications in the test (Table 3). For all traits the year was statistically significantly different according to the ANOVA, except NAWF2, which found 2017 and 2019 to be statistically similar (Table 6). For all of the parameters, location and genotype were statistically significant. Year was statistically significant for all of the variables except for NACB1. (Table 4) The correlations were combined over years and locations because of this study wanted to get to the whole picture of the maturity of the genotypes, over a wide range of planting locations and environments. Additionally, by combining by year and location, the locations with only two reps were able to be utilized in this study.

Node of First Fruiting Branch, NFFB

ANOVA results for NFFB indicated statistical significance for the variables of genotype, year, location, and interactions between genotype x year. Rep, genotype x year, and genotype x location x year were not found to be statistically significant (Table 4). This measurement had the lowest CV tested in this research. The H^2 was 0.90, so it is highly heritable. (Table 3) Node of first fruiting branch was the method that found the most statistical differences among the individual genotypes (Table 7). Some locations had a wider range of NFFB. HGA had the highest mean, and BGA the lowest (Figure1).

The first Measurement of Node above White Flower, NAWF1

The first NAWF rating was collected at or closely to 60 days after planting. ANOVA results showed the first NAWF rating was significant by rep, genotype, year, location, and genotype x location with a p value less than or equal to .0001 (Table 4). Each year was found to be statistically different to each other (Table 6). In this test, NAWF1 had a CV of 8.03 and a H^2 of 0.86, which is highly heritable (Table 3). Tukey's HSD for NAWF1 found statistically significant difference between genotypes, with FM954GLT being statistically fuller season than the other genotypes (Table 8). Some locations had a very wide range of values for NAWF1. LGA had a much higher range of values than AGA or MVGA. BGA and VGA had the lowest means for NAWF1. (Figure 2)

The second measurement of Node above white Flower, NAWF2

The second measurement of node above white flower was collected at approximately 74 DAP. The ANOVA indicated NAWF2 was statistically significant by rep, genotype, year, and location. Genotype x location was significant with a p value of .0004, but genotype x year was not significant with a p value of .0905. (Table 4) NAWF2 had a CV of 11.22 and a H^2 of 0.69 (Table

3). This measurement found 2017 and 2019 to not be statistically different from each other, but different than 2018 (Table 6). This measurement found statistical differences among genotypes, with FM954GLT being statistically the longest season genotype, and DG3385B2XF, ST4747GLB2, ST4949GLT, ST4946GLB2, and PHY333WRF being statistically the same as the earliest varieties (Table 8). Most of the locations were similar for NAWF2. SGA and VGA had lower means than the other locations. (Figure 3)

The first measurement of Node Above Cracked Boll, NACB1

The ANOVA demonstrated the NACB1 count was statistically significant by rep, genotype, year, and location. The interaction of genotype x location was significant with p=0.0159. There was no statistical significance in genotype x year. (Table 4) The first and second NACB ratings were highly correlated, as indicated by a r value of 0.817 with p values less than 0.0001 (Table 5). NACB1 had the highest broad sense heritability (H^2=0.99) among all tested variables (Table 3). The first node above cracked boll rating found each year to be statistically different from the other years (Table 6). This maturity method found FM954GLT to be statistically different as the fullest maturity variety (Table 9). There was a large difference in values for NACB1 at different locations. MGA had the largest range of values. LGA had the lowest mean as well as a small range of values. (Figure 2)

The second measurement of Node Above Cracked Boll, NACB2

For this parameter, a smaller number usually indicates an earlier variety. In the two weeks between counts, boll opening advanced at least four nodes. NACB2 was statistically significant by rep, genotype, year, and location (Table 4). NACB2 a CV of 11.19 and a H^2 of 0.69 (Table 3). This measurement found each year to be statistically different from the other years (Table 6). NACB2 found FM954GLT to be statistically different as the fullest maturity variety.

PHY333WRF, ST4949GLT, and ST4747GLB2 were found to be statistically the earliest varieties (Table 9). The second rating of node above cracked boll had inconsistent values at the different locations. AGA had both the highest mean and range of values. (Figure 2)

The First measurement of Percent Open, Open1

The higher the value for Open1, the earlier the variety is. Open1 is statistically significant by rep, genotype, year, location, and interactions of genotype x location x year. The first rating of percent open has a positive and significant correlation coefficient (r = 0.533) to Open2, but negatively correlated to the first NAWF rating (r = -0.535). Of all the other parameters measured, NFFB was most highly correlated to Open1, with an r of -0.374 (Table 5). Open1 had a CV of 25.04 (Table 3). The first percent open rating found each year to be statistically different from the other years (Table 6). This measurement found FM954GLT, BASF-10, ST5517GLT, BASF-09, DP1252B2F, and PHY444WRF to be statistically the fullest maturing genotypes. Open1 found PHY333WRF, ST4949GLT, and ST4747GLB2 to be statistically the earliest genotypes (Table 10). For Open1, VGA and BGA had the highest range of values (Figure 4).

The Second measurement of Percent Open, Open2

Open2 was statistically significant by genotype, year, and location. Just like Open1, Open2 indicates an earlier maturity variety when the rating is higher. The second rating of percent open was highly correlated to both NAWF1 and Open1 (Table 5). This maturity method had a CV of 23.29, and a H^2 of 0.61 (Table 3). Open2 found 2018 and 2019 statistically different (Table 6); this measurement was not collected in 2017. The second Open measurement found statistical differences among varieties (Table 10). For Open2, SGA had the lowest range of values (Figure 5).

Yield

The ANOVA indicated yield was statistically significant for rep, genotype, year, and location with a p value less than .0001 (Table 6). Yield had a CV of 14.22 and a H^2 of 0.54 (Table 3). The genotypes PHY444WRF, ST6182GLT, DP1646B2XF, DP1252B2F, and ST4946GLB2 have the highest yield over all three years. The varieties with the lowest yield over all the years were DG3385B2XF, BASF-09, ST4949GLT, BASF-10, CG3885B2XF, and NG5007B2XF (Table 11). The location with the highest mean yield was LGA. MGA had the lowest mean yield. (Figure 6).

Means Comparisons by Maturity Groupings

There are some differences among the groupings of early, mid, and full varieties by the maturity methods. For the maturity methods NACB1, Open1, NAWF1, NACB2, and Open2, mid maturing varieties were statistically the same as full and early, but the early group was different than the full maturity group. There were no statistical differences among the maturity groupings for Yield and NFFB. The Full grouping is statistically different than mid and early for NAWF2. (Tables 12 and 13)

Discussion

Although the feasible planting date range for the region is April 1 through May 25, with early April planting uncommon, little yield difference has been shown within this planting window. Some planting dates for this study are after this window, as seen in Tables 1 and 4. Whitaker et al. (2019) explains the main risk with late plantings are delayed and inadequate crop maturity but can be mitigated with irrigation to prevent the need for replantings, which may not be possible if the first planting is late. All fields in this study were under center pivot irrigation and had good stands. No replantings were necessary, so the planting dates just outside the normal planting

window most likely caused no issue. The range of NFFB was 4.7 to 8, which is a reasonable range, as it is similar to the range reported by Babar et al. in 2002 (Table 2). The H^2 found for NFFB in this research is in line with Hougni et al. (2017) found (Table 3). This was confirmed by Gwathmey et al. as they found maturity methods collected before flowering to have a lower heritability (2016). Additionally, both this research and Hougni et al. found NFFB to have the lowest CV of all the measured methods, even though the two studies compared NFFB to different methods (2017) (Table 3).

When compared with the other tested morphological methods, NFFB showed the most statistical separation among individual genotypes. ST5517GLTP and ST4747GLB2 were the genotypes with the highest mean of NFFB and were statistically different than most of the other genotypes. The genotypes NG5007B2XF, DG3385B2XF, PHY333WRF, and BASF-09 had the lowest NFFB mean (Table 7). Although NFFB found more statistical difference between individual genotypes, it did not find any statistically significant difference between the genotypes when they were grouped into their maturity groupings. (Tables 12 and 13) NFFB was not highly correlated to any of the other methods. NFFB was most correlated to the Open1, with a correlation coefficient of -.374 (Table 5).

When comparing maturity groupings across the maturity methods, almost all methods indicated significant difference between the full and early genotype groupings (Tables 12 and 13). NAWF2 was the maturity measurement most highly correlated with Yield, even though its correlation was only -0.3541 (Table 5). NAWF2 was also the only method that found the full grouping statistically different from both the mid and early maturity groupings. (Table 13)

The highest absolute value of correlation coefficient between parameters was NACB1 and NACB2 (Table 5). This is reasonable as they’re the same measurement taken at different

times. The second highest absolute value of correlation overall was NAWF1 and Open2 (-.637, p<.0001) (Table 5). This gives credence to the researcher being able to visually estimate the percent of bolls that are open, instead of taking the time-consuming measurement of NAWF. NAWF1 was also highly correlated to Open1, further confirming this. (Table 5)

The parameter most closely correlated with yield, with an r of -.354 was NAWF2 (Table 5). The timing of NAWF2 is the suggested optimal timing of NAWF as described by Waddle (1974). Due to NAWF2 being the most correlated with yield, and being consistent with prior research's timing, this research suggests NAWF2 being the best NAWF timing. Open1 was the only parameter measured that was significant for genotype x location x year (Table 4). This gives more credence to its ability to be a valuable maturity method, even though it had the highest CV (Table 3). However, the CV for Open1 was only 25, which is below the critical value of 30, so it is considered in an acceptable range (Table 3) (Hougni et al, 2017). Open1 at HGA, LGA, and SGA, and Open2 at SGA had low ranges of values which suggests this measure was not taken at the optimal time for these locations (Figures 6 and 7). Due to varying rates of growth at different locations, this research recommends taking Open, not based on DAP, but when there is the most variability of Open in the field.

NAWF1, NAWF2, NACB1, NACB2, and yield were significant by rep. This may be explained by environmental and soil differences. NACB1 and NACB2 were highly correlated to each other (.817, p < .0001) (Table 5). This suggests they are redundant. It is not beneficial to take this reading more than once. Open1 and Open2 have a correlation coefficient of .533 (Table 5). The most highly heritable (broad sense heritability) maturity methods were NFFB, NACB1, and NAWF1 (Table 3). ST4747GLT and ST4949GLT had earlier NAWF1, NAWF2, NACB1, NACB2, and Open1 ratings (Tables 8, 9, and 10). The genotype consistently ranked as the fullest

among most maturity methods was FM954GLT. It had a higher first and second ratings of NAWF and NACB, as well as lower Open1 and Open2. (Tables 8, 9, and 10) This confirms what is known about its fullness as a cultivar.

For Open1, at the locations where there is no statistical difference among varieties, the difference between the means of the latest and earliest genotypes were small. This is because the timing of the taking of Open is paramount. It is most useful when the variance among varieties is the greatest, which can be difficult to predict due to environmental factors, such as accumulated heat units and precipitation. DAP can be an indicator of when to begin to check the field for the timing of Open, but the actual date of rating may need to be delayed until the timing is correct. As there is no published broad-sense heritability for a visual estimate of the percent open of cotton, it must be compared with its heritability in this study to the other maturity methods, and their published heritability. Yield had the lowest heritability of all measured variables. However, since this is lint yield, and not seedcotton yield, the value from this study is a high heritability compared to other published values (Zeng and Pettigrew, 2005). Open2 had a similar H^2 as NACB2 and NAWF2 (Table 3). A visual estimation, when taken correctly, is a quality morphological measure of maturity and has high heritability.

The reason the different maturity methods did not have higher correlations is because they are measuring different parts of what makes up cotton maturity. NFFB measures how soon a plant switches its focus from vegetative to reproductive growth. NAWF tells when the plant begins to produce reproductive structures. NACB and Open indicate how quickly the plant can make, and fill a boll, as well as how quickly bolls open. Each is an important part of the overall maturity process, but don’t measure the exact same processes.

Conclusion

Properly knowing a genotype's maturity assists in proper crop termination and harvest scheduling. NFFB was found to be a highly heritable trait in this study and consistent with other research's findings but was not correlated to any of the other tested maturity methods. As a maturity parameter, considering the time it takes to collect and its high correlation to more traditionally trusted maturity methods, a correctly timed and collected Open can be a useful tool. Since Open is most valuable when taken when the disparity between the varieties is the greatest, DAP was not an optimal way of planning when this rating was to be taken. Open2 was highly correlated to NAWF1. With NAWF being an accepted maturity method, their high correlation suggests the credibility of Open. The practical application of Open can be useful in testing and breeding environments, where large numbers of varieties need to have maturity taken quickly and reliably. It would be more useful in collecting different methods than the same method multiple times. No single maturity method can be used as a sole indicator of maturity of the crop. Collecting several maturity methods at different crop stages can give a more exhaustive picture of a specific genotype's maturity and help the breeder or farmer grow the genotype to its fullest yield potential. This research herein recommends that researchers continue examining cotton maturity methods and come to an agreement of in season and post season methods that work together in determining a crop's maturity

Tables

Table 1. Planting location, planting date, harvest date, and soil type by year

Year	Loc	Near	Row spacing (in)	Soil type	# reps	Planting Date	Harvest Date
2017	VGA	Vienna, GA	38	Faceville fine sandy loam with 0-2% slopes	3	10 May 2017	6 June 2017
	AGA	Sasser, GA	38	Tifton loamy sand with 0-2% slopes	3	11 May 2017	18 Nov. 2017
	MVGA	Midville, GA	38	Tifton loamy sand with 2-5% slopes	3	6 May 2017	16 Oct. 2017
	MGA	Mitchell County, GA	36	Norfolk Loamy sand with 2-5% slopes	3	9 June 2017	13 Nov. 2017
2018	BGA	Baxley, GA	38	Tifton loamy sand with 2-5% slopes	3	3 May 2018	4 Nov.2018
	VGA	Vienna, GA	38	Norfolk loamy fine sand with 2-5% slopes	3	9 May 2018	not harvested
	MGA	Mitchell County, GA	36	Norfolk Loamy sand with 2-5% slopes	2	19 May 2018	not harvested
	SGA	Sycamore, GA	36	Tifton loamy sand with 2-5% slopes	2	7 June 2018	14 Nov. 2018
	LGA	Leary, GA	36	Wagram loamy sand with 0-5% slopes	3	6 June 2018	not harvested
2019	BGA	Baxley, GA	38	Tifton loamy sand with 2-5% slopes	2	8 May 2019	24 Oct. 2019
	LGA	Leary, GA	36	Lucy loamy sand with 0-5% slopes	2	15 May 2019	11 Oct. 2019
	VGA	Vienna, GA	38	Norfolk loamy fine sand with 2-5% slopes	2	21 May 2019	11 Nov. 2019
	HGA	Dawson, GA	38	Greenville sandy loam with 2-5% slopes	3	30 May 2019	25 Nov. 2019

Table 2. Range and Mean by Year for different variables

		NFFB	NAWF1	NAWF2	NACB1	NACB2	Open1	Open2	Yield
							%		Lint lbs./ acre
Range	All Years	4.7 - 8	3 - 12	.5 - 6.9	5.4 - 26	2.9 - 20	1 - 90	5 - 90	539 - 2313
	2017	4.9 - 7.6	3.5 - 11	.5 - 6.4	9 - 23	5.6 - 20	5 - 90	–	551 - 1706
	2018	5.2 - 7.8	4.1 - 12	2 - 6.5	5.4 - 26	2.9 - 12.7	1 - 70	5 - 70	539 - 2050
	2019	4.7 - 8	3 - 10.3	1.2 - 6.9	6.3 - 18.9	5.6 - 14	5 - 90	10 - 90	566 - 2313
Mean	All Years	6.05	6.77	4.16	13.81	9.43	30.51	44.44	1256
	2017	5.87	6.41	4.18	16.18	10.31	45.10	–	1079
	2018	6.35	7.13	3.81	12.42	7.91	16.51	39.59	1355
	2019	6.07	6.99	4.25	12.09	9.20	29.62	50.10	1435

Table 3. Number of observations, Coefficient of Variation, and Heritability estimates

	NFFB	NAWF1	NAWF2	NACB1	NACB2	Open1	Open2	Yield
N[a]	428	413	366	371	254	367	208	301
CV[b]	6.54	8.03	11.22	8.53	11.19	25.04	23.29	14.22
H2[c]	0.90	0.86	0.69	0.99	0.69	–	0.61	0.54

[a] Abbreviation; N, Number of observations (plots)

[b] Abbreviation; CV, Coefficient of Variation

[c] Abbreviation; H2, Heritability (broad sense)

Table 4. Analysis of variance for each variable

NFFB

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	17	3.02738681	0.1780816	1.14	0.3209
Genotype	15	33.3449162	2.2229944	14.17	<.0001
Year	2	11.1721927	5.5860964	35.61	<.0001
Location	7	25.3295768	3.6185110	23.06	<.0001
Genotype*Year	28	4.417061	0.1577522	1.01	0.4625
Genotype*Location	101	30.1591258	0.2986052	1.9	<.0001
Genotype*Location*Year	13	2.93671092	0.2259008	1.44	0.1417

NAWF1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	17	21.327659	1.254568	4.24	<.0001
Genotype	15	55.5191709	3.701278	12.52	<.0001
Year	2	78.9919216	39.495961	133.63	<.0001
Location	7	515.322762	73.617537	249.07	<.0001
Genotype*Year	28	9.406914	0.335961	1.14	0.2973
Genotype*Location	100	53.7128155	0.537128	1.82	0.0001
Genotype*Location*Year	13	4.8358521	0.371989	1.26	0.2397

NAWF2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	14	22.0212843	1.5729489	7.22	<.0001
Genotype	15	33.3215371	2.2214358	10.19	<.0001
Year	2	13.1239286	6.5619643	30.11	<.0001
Location	7	374.371192	53.4815989	245.39	<.0001
Genotype*Year	26	8.0970714	0.3114258	1.43	0.0905
Genotype*Location	99	38.2058028	0.3859172	1.77	0.0004
Genotype*Location*Year	0	0	.	.	.

NACB1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	16	85.161636	5.322602	3.83	<.0001
Genotype	15	336.662196	22.444146	16.15	<.0001
Year	2	12.703137	6.351568	4.57	0.0115
Location	7	2140.63349	305.804807	220.03	<.0001
Genotype*Year	28	28.504925	1.018033	0.73	0.8345
Genotype*Location	100	200.389176	2.003892	1.44	0.0159
Genotype*Location*Year	8	21.794253	2.724282	1.96	0.0534

NACB2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	10	228.468526	22.8468527	20.49	<.0001
Genotype	15	139.090731	9.2727154	8.32	<.0001
Year	1	52.152381	52.152381	46.77	<.0001
Location	4	987.358228	246.839557	221.38	<.0001
Genotype*Year	13	18.335619	1.4104322	1.26	0.2416
Genotype*Location	58	87.3612909	1.5062292	1.35	0.0796
Genotype*Location*Year	0	0	.	.	.

Open1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	14	1676.55526	119.75395	2.05	0.0159
Genotype	15	7817.08329	521.13889	8.93	<.0001
Year	2	27342.5553	13671.2776	234.27	<.0001
Location	6	42511.4914	7085.24858	121.41	<.0001
Genotype*Year	28	1524.73196	54.45471	0.93	0.5666
Genotype*Location	86	8248.22048	95.90954	1.64	0.0024
Genotype*Location*Year	15	3836.05247	255.73683	4.38	<.0001

Open2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	8	1604.83305	200.60413	1.87	0.0717
Genotype	15	10209.6860	680.64574	6.35	<.0001
Year	1	7952.08468	7952.08468	74.19	<.0001
Location	4	50197.3188	12549.3297	117.09	<.0001
Genotype*Year	13	983.2967	75.63821	0.71	0.7542
Genotype*Location	56	10722.5042	191.47329	1.79	0.005
Genotype*Location*Year	0	0	.	.	.

Yield

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	12	3284908.27	273742.36	8.57	<.0001
Genotype	15	2797530.34	186502.02	5.84	<.0001
Year	1	603709.17	603709.17	18.9	<.0001
Location	5	13873145.1	2774629.03	86.87	<.0001
Genotype*Year	13	559680.47	43052.34	1.35	0.1901
Genotype*Location	69	2951752.34	42779.02	1.34	0.0672
Genotype*Location*Year	0	0	.	.	.

DF = Degrees of Freedom
SS = Sum of Squares

Table 5. Pearson correlation coefficients among eight measured traits

	NFFB	NAWF1	NAWF2	NACB1	NACB2	Open1	Open2	Yield
NFFB	1	0.254[a]	0.205[a]	-0.227[a]	-0.230[b]	-0.374[a]	-0.0301	0.0456
NAWF1		1	0.308[a]	-0.190[b]	-0.347[a]	-0.534[a]	-0.637[a]	-0.112
NAWF2			1	0.011	-0.236[b]	-0.169	0.273	-0.354[a]
NACB1				1	0.8170[a]	0.290[a]	-0.250[b]	-0.097
NACB2					1	0.266[b]	-0.510[a]	0.166
Open1						1	0.533[a]	0.109
Open2							1	-0.089
Yield								1
p-values	a	<.0001		b	<=.0005			

Table 6. Comparison of means among years by parameter

Year		NFFB	NAWF	NAWF2	NACB1	NACB2	Open1	Open2	Yield
2017	Mean	5.87	6.41	4.18	16.19	10.31	45.10	-	1079.9
		C	C	A	A	A	A	-	C
2018	Mean	6.07	7.13	3.81	12.42	7.91	16.51	39.59	1355.1
		A	A	B	B	C	C	B	B
2019	Mean	6.35	6.99	4.25	12.09	9.20	29.62	50.10	1435.8
		B	B	A	C	B	B	A	A

Means with the same letter are not significantly different at P=0.05 by Tukey's HSD.

Table 7. Comparison of NFFB means among tested genotypes

Genotype	Mean	
ST5517GLT	6.7	A
ST4747GLB2	6.5	AB
PHY444WRF	6.5	BC
FM954GLT	6.3	CD
ST4946GLB2	6.3	CDE
DP1646B2XF	6.1	DEF
CG3885B2XF	6.1	EFG
DP1252B2F	6.0	FGH
ST6182GLT	5.9	FGHI
DP1538B2XF	5.9	GHIJ
BASF-10	5.9	GHIJ
ST4949GLT	5.9	GHIJ
BASF-09	5.8	HIJK
PHY333WRF	5.8	IJK
DG3385B2XF	5.7	JK
NG5007B2XF	5.6	K

Means with the same letter are not significantly different at P=0.05 by Tukey's HSD.

Table 8. Comparison of NAWF Means among tested genotypes

	NAWF1		NAWF2	
Genotype	Mean		Mean	
FM954GLT	7.9	A	4.9	A
ST6182GLT	7.2	B	4.3	BCDE
DP1646B2XF	7	BC	4.5	BC
BASF-10	7	BC	4.6	B
DP1252B2F	6.9	BCD	4.4	BCD
BASF-09	6.9	CDE	4.4	BC
NG5007B2XF	6.8	CDEF	4.3	BCDE
PHY444WRF	6.8	CDEF	4.1	E
ST5517GLT	6.8	CDEF	4.1	DE
CG3885B2XF	6.6	DEFG	4.2	CDE
DG3385B2XF	6.6	EFGH	3.8	F
ST4949GLT	6.5	FGH	3.7	F
PHY333WRF	6.4	GH	3.6	F
DP1538B2XF	6.4	GHI	4.1	DE
ST4747GLB2	6.3	HI	3.8	F
ST4946GLB2	6.1	I	3.7	F

Means with the same letter are not significantly different at P=0.05 by Tukey's HSD.

Table 9. Comparison of NACB means by genotype

	NACB1		NACB2	
Genotype	Mean		Mean	
FM954GLT	16.4	A	11.6	A
DP1646B2XF	14.7	B	9.8	BC
BASF-10	14.6	BC	9.9	BC
DP1252B2F	14.2	BCD	9.8	BC
PHY444WRF	14.2	BCDE	9.2	CD
DG3385B2XF	14	CDE	9.2	C
CG3885B2XF	13.9	DEF	9.3	C
BASF-09	13.8	DEF	10.4	B
NG5007B2XF	13.7	DEF	9.5	C
ST5517GLT	13.6	DEF	9.4	C
ST6182GLT	13.6	EFG	9.2	C
DP1538B2XF	13.3	FG	9.7	BC
ST4946GLB2	13.3	FG	9.3	C
PHY333WRF	12.9	GH	8.4	DE
ST4747GLB2	12.6	H	8.1	E
ST4949GLT	12.3	H	8.3	E

Means with the same letter are not significantly different at P=0.05 by Tukey's HSD.

Table 10. Comparison of Open means among tested genotypes

	Open1		Open2	
Genotype	Mean		Mean	
PHY333WRF	39	A	61.5	A
ST4747GLB2	36.7	AB	53.6	AB
ST4949GLT	36.1	AB	48.6	BCD
CG3885B2XF	33.9	BC	37.5	FGH
ST4946GLB2	33.7	BCD	49.3	BCD
NG5007B2XF	33.3	BCD	49.7	BC
DG3385B2XF	32.8	BCD	46.4	BCDE
DP1646B2XF	31.5	CDE	41.1	DEFGH
DP1538B2XF	30.5	CDEF	44.3	CDEF
ST6182GLT	29.3	DEFG	36.5	FGH
PHY444WRF	27.4	EFGH	41.9	CDEFG
DP1252B2F	26.4	FGH	39.6	EFGH
BASF-09	25.9	GH	44.3	CDEF
ST5517GLT	25.2	GH	46.2	BCDE
BASF-10	24.6	H	35.7	GH
FM954GLT	23	H	32.9	H

Means with the same letter are not significantly different at P=0.05 by Tukey's HSD.

Table 11. Comparison of Yield means among tested genotypes

Genotype	Mean	
PHY444WRF	1462	A
ST6182GLT	1377	AB
DP1646B2XF	1367	ABC
DP1252B2F	1358	ABC
ST4946GLB2	1350	ABCD
ST4747GLB2	1279	BCDE
DP1538B2XF	1259	CDE
PHY333WRF	1234	DE
ST5517GLT	1232	E
FM954GLT	1222	E
NG5007B2XF	1186	EF
CG3885B2XF	1176	EF
BASF-10	1173	EF
ST4949GLT	1164	EF
BASF-09	1099	F
DG3385B2XF	1090	F

Means with the same letter are not significantly different at P=0.05 by Tukey's HSD.

Table 12. Genotypes grouped by their company described maturity

Early	Mid	Full
DG3385B2XF	CG3885B2XF	BASF-09
PHY333WRF	NG5007B2XF	BASF-10
ST4747GLB2	PHY444WRF	DP1252B2F
ST4946GLB2	ST4949GLT	DP1538 B2XF
	ST5517GLT	DP1646B2XF
		FM954GLT
		ST6182GLT

Table 13. Means of tested maturity methods by genotype's maturity classification

	Maturity	Mean	
NFFB			
	Full	5.98	A
	Mid	6.13	A
	Early	6.09	A
NAWF1			
	Full	7.03	A
	Mid	6.72	AB
	Early	6.32	B
NAWF2			
	Full	4.45	A
	Mid	4.07	B
	Early	3.70	B
NACB1			
	Full	14.37	A
	Mid	13.51	AB
	Early	13.15	B
NACB2			
	Full	10.04	A
	Mid	9.11	AB
	Early	8.70	B
Open1			
	Full	27.30	A
	Mid	31.21	AB
	Early	35.75	B
Open2			
	Full	53.43	A
	Mid	45.55	AB
	Early	39.27	B
Yield			
	Full	1263.1	A
	Mid	1257.6	A
	Early	1247.1	A

Levels not connected by same letter are significantly different at P=0.05 by Tukey's HSD.

Figures

Figure 1. Distribution of NFFB by Location

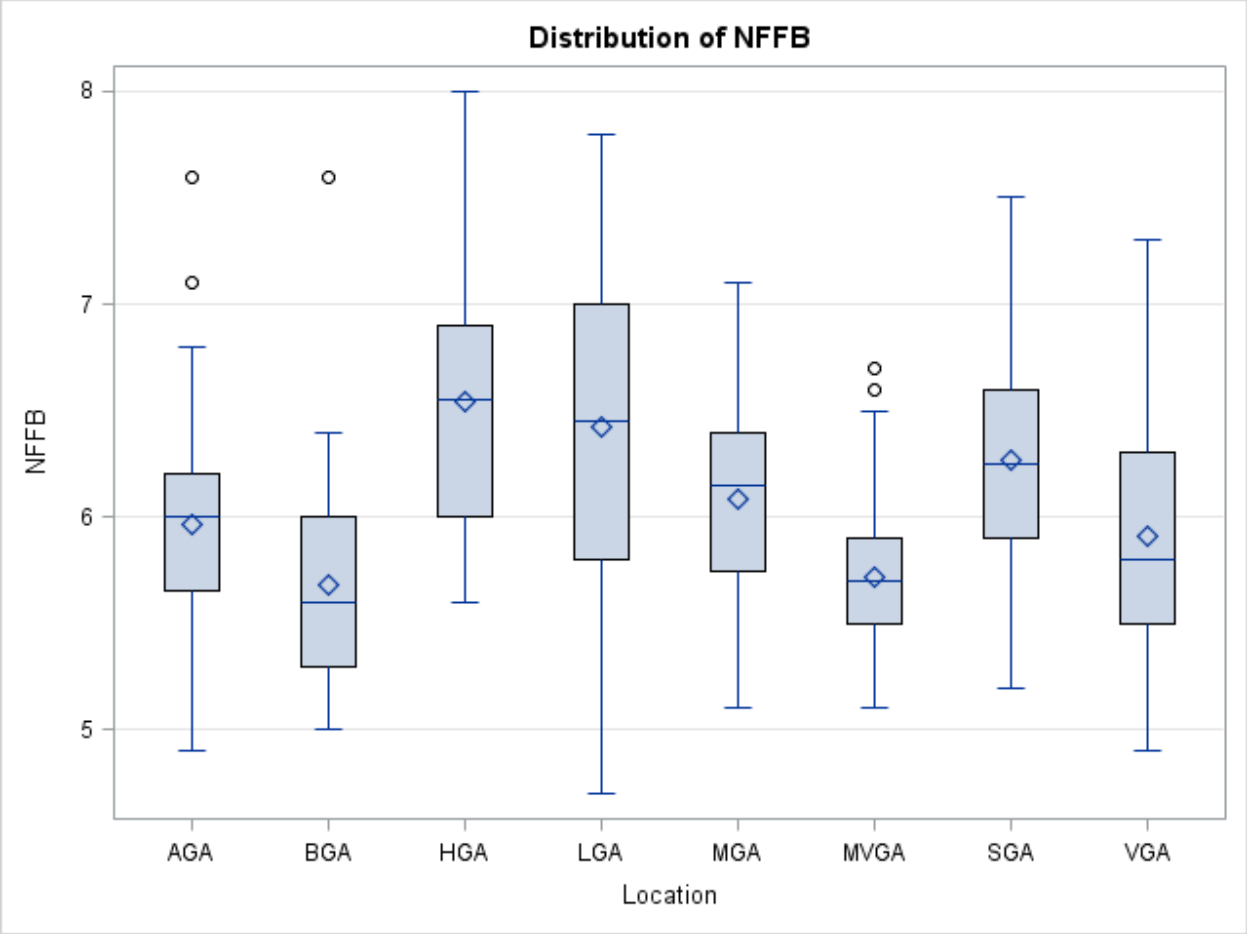


Figure2. Distribution of NAWF1 by Location

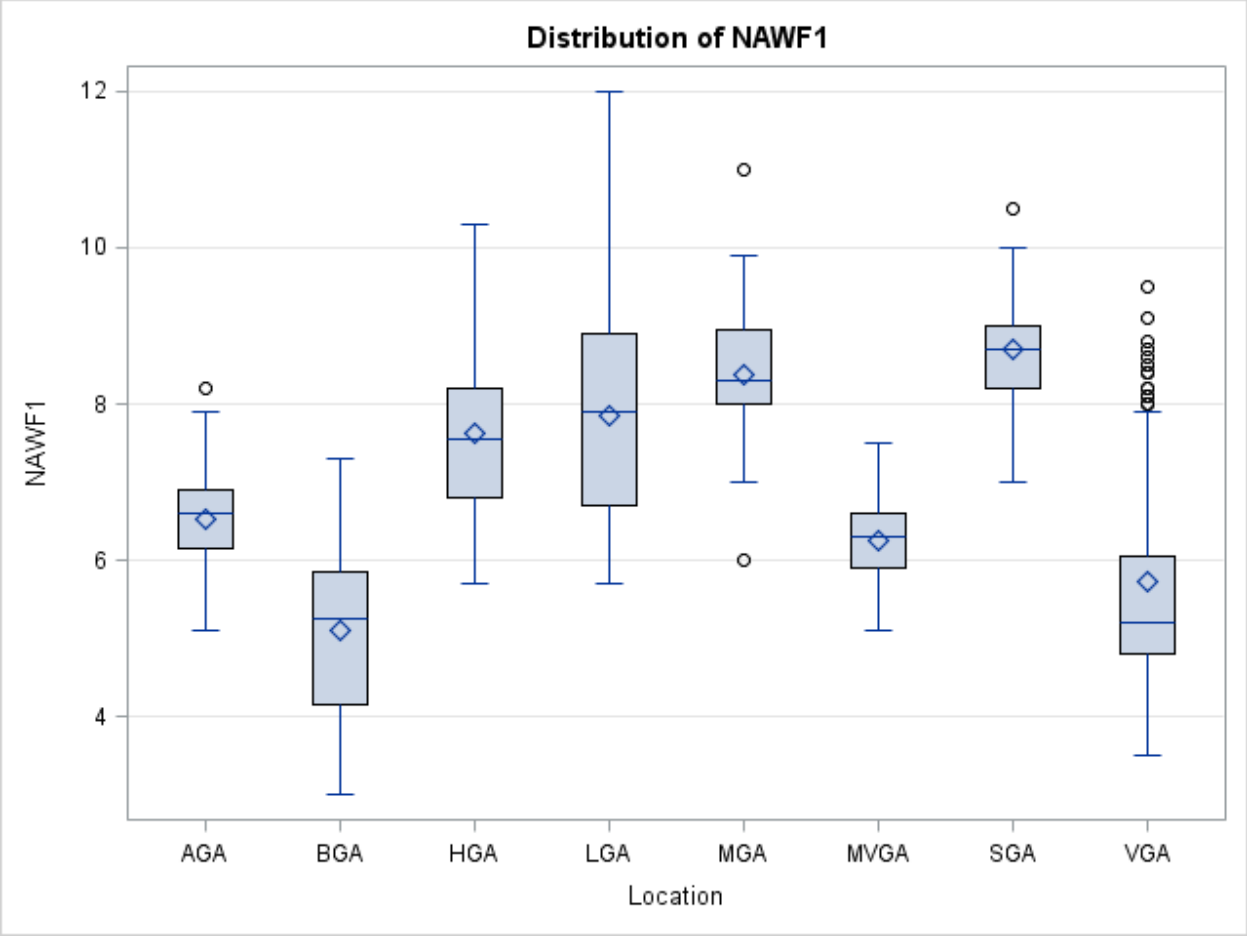


Figure3. Distribution of NAWF2 by Location

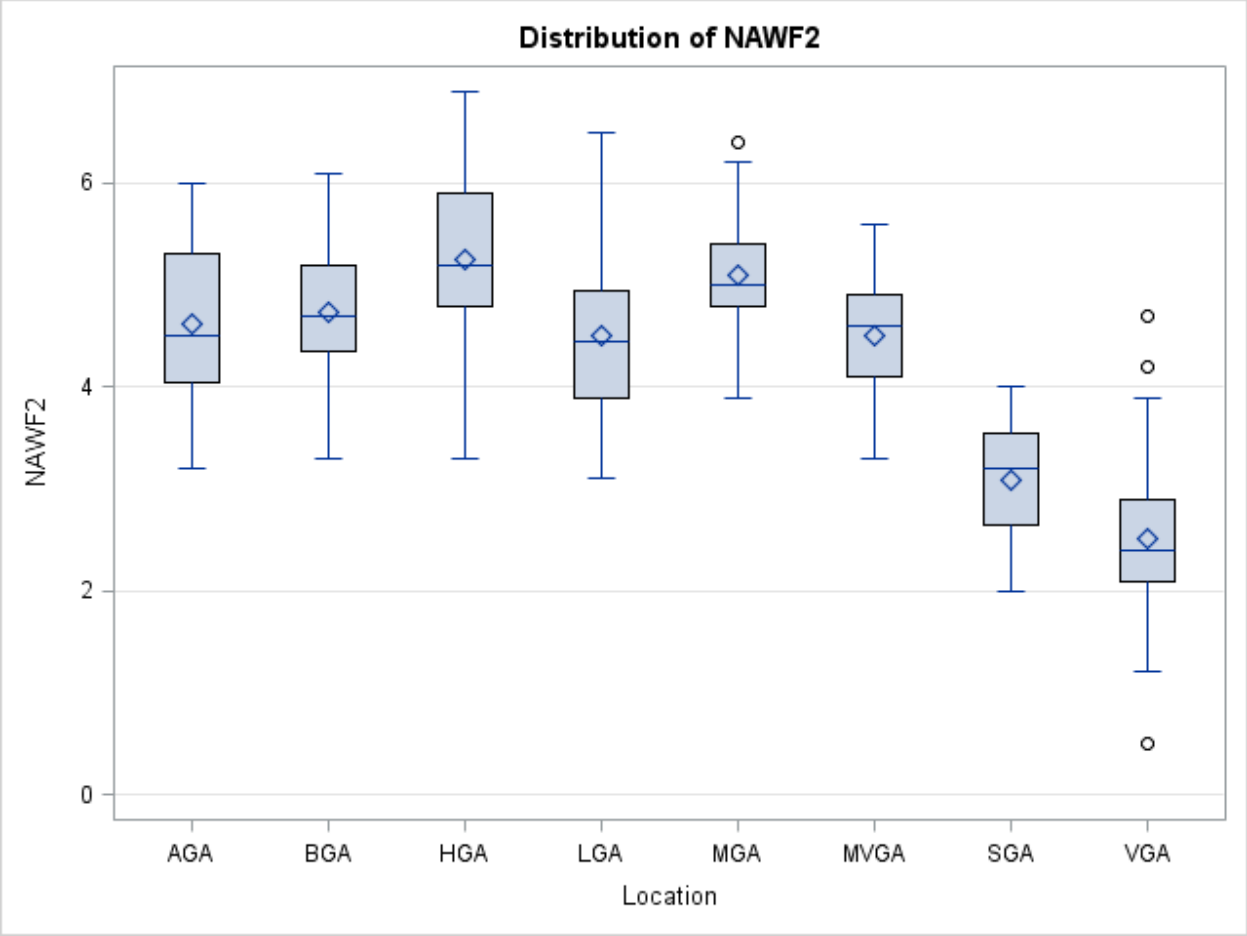


Figure4. Distribution of NACB1 By Location

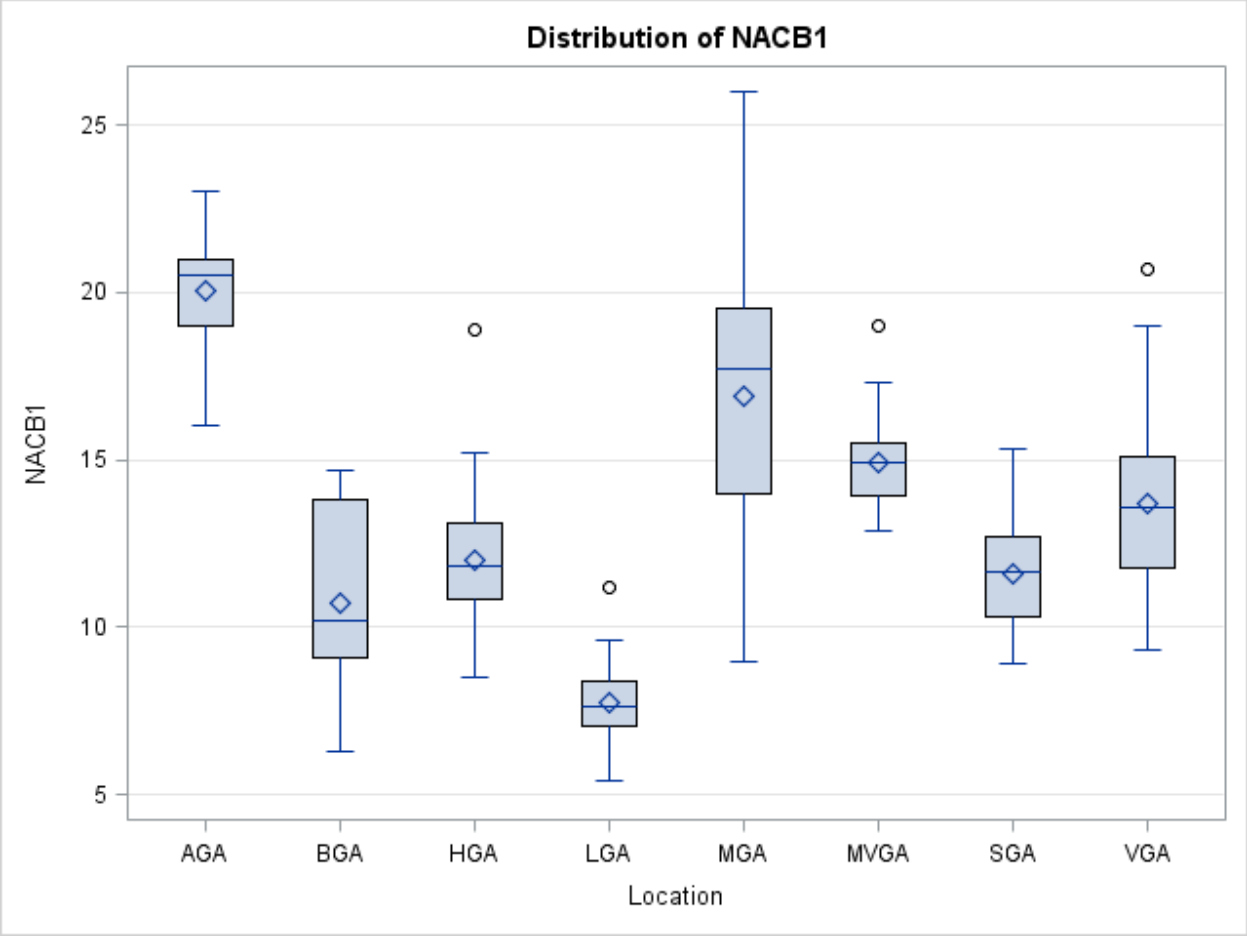


Figure 5. Distribution of NACB2 by Location

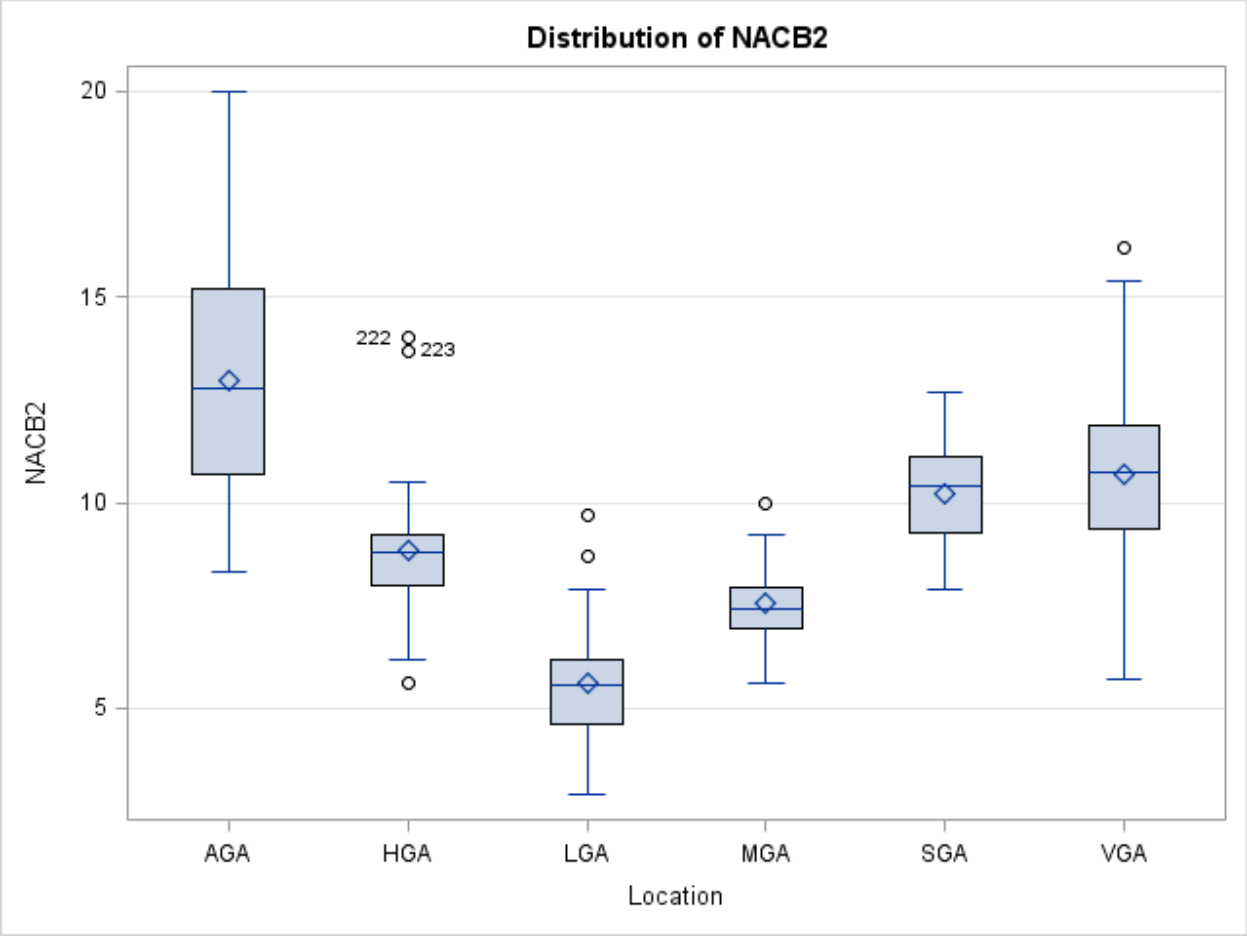


Figure 6. Distribution of Open1 by Location

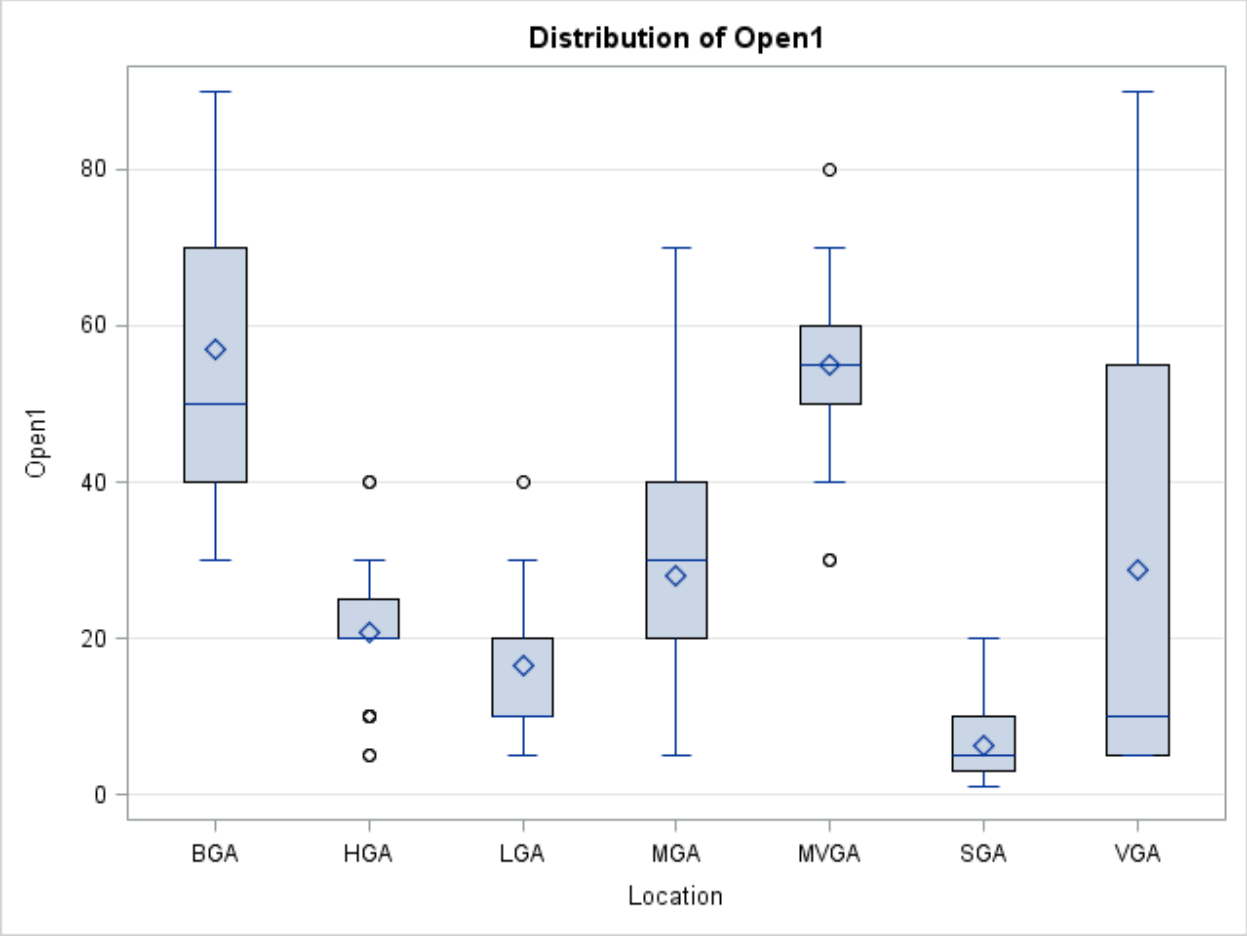


Figure 7. Distribution of Open2 by Location

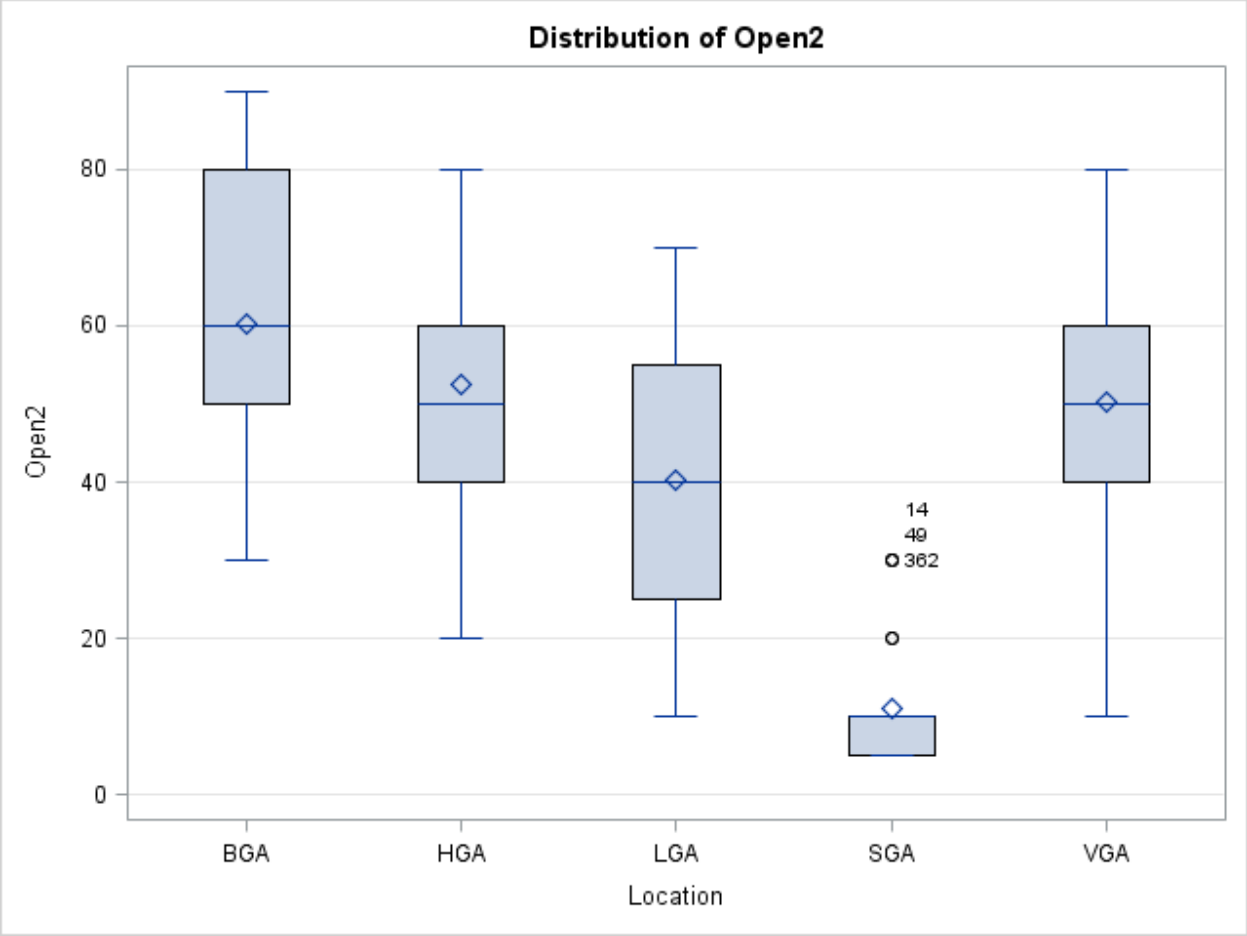
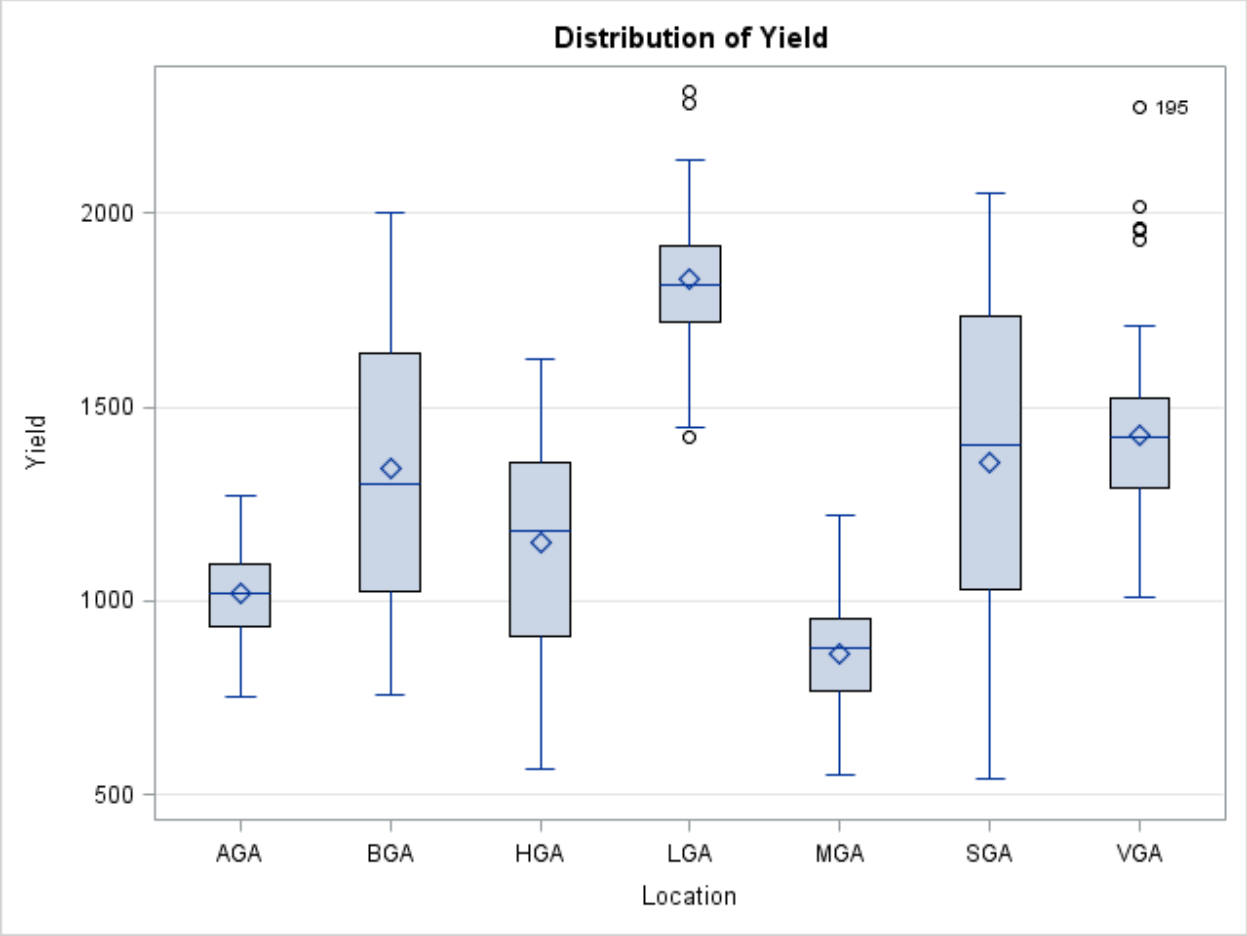


Figure 8. Distribution of Yield by Location



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