RECURRENT PHENOTYPIC SELECTION FOR INCREASED

WINTER PRODUCTIVITY IN ANNUAL RYEGRASS

(*LOLIUM MULTIFLORUM* LAM.)

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RECURRENT PHENOTYPIC SELECTION FOR INCREASED WINTER PRODUCTIVITY IN ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM* LAM.)

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THESIS ABSTRACT

RECURRENT PHENOTYPIC SELECTION FOR INCREASED WINTER PRODUCTIVITY IN ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM* LAM.)

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In the Southeastern USA, dry matter distribution of cool season annual and perennial grasses is concentrated more in spring months with relatively less production in late autumn and early winter. Annual cool season grasses have the tendency for earlier spring production than perennial grasses. Due to lower availability of forage of both types of cool season grasses in early winter, animals are typically fed stored forages in order to meet their nutritional requirement, which results in increased management costs. A preferable approach is to provide live forage for maximizing the forage intake by animals and minimizing feeding of stored forages and grains. Annual ryegrass

(*Lolium multiflorum* Lam.) is a cool season annual crop that originated in Southern Europe. The growing season for annual ryegrass in the southeastern USA is from late summer to late spring with maximum dry matter production during the spring months. Phenotypic recurrent selection was used to increase early winter dry matter production. The base population (C_0) was generated from a polycross nursery of an equal number of plants (50) from the five top performing cultivars in Alabama Annual Ryegrass Trials along with cv. Gulf. These plants were open pollinated and bulk harvested for two years to create a random mating population. Selection was carried for two cycles (C_1 and C_2).The objectives of this study were a) to select for increased winter production; b) to study correlated responses; c) to evaluate the first two cycles of phenotypic recurrent selection and d) to determine the relationship between dry matter yield and indirect responses. For the first objective, evaluation and selection with grid restriction followed by recombination was carried out for two years. Selection was done on the basis of individual plant dry matter yield, individual plant green matter yield, visual appearance, individual plant dry matter percentage, and a random selection. After two cycles of selection, correlated responses such as seed yield, maturity, plant erectness and disease incidence, were also collected. Seed of C_0, C_1 and C_2 along with check cultivars was used for solid-seeded plot evaluation in five environments in Alabama in 2008/09. Correlated responses have shown remarkable change from C_0 to C_1 but remained constant from C_1 to C_2. Selection resulted in increased early winter dry matter production at three out of five evaluation locations. Indirect responses (green matter, visual appearance and percent dry matter) have shown insignificant differences from dry matter yield, the direct response.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. LITERATURE REVIEW

Species Description

Annual ryegrass (*Lolium multiflorum* Lam.), also known as Italian ryegrass, is a cool season annual bunchgrass that originated in Southern Europe (Jung et al., 1996) and belongs to the Poaceae family. In its natural state it is a diploid (2n = 2x = 14) but tetraploids (2n = 4x = 28) have been produced artificially by colchicine treatment (Ahloowalia, 1967). As the common name indicates, its life cycle is that of an annual, but in temperate regions it can behave as biennials or short duration perennials. It is a cross pollinated crop due to gametophytic self incompatibility which is controlled by two multi-allelic genes S and Z (Fearon et al., 1983). Percentage of self compatibility is 7.76% (Arcioni and Mariotti, 1983). It is interfertile with *Lolium perenne* L. and *Festuca* sp. (Jauhar, 1975). Its growth is favored under fertile and well drained soils, but it can survive on poorly drained soils. The best soil pH range is from 5.5 to 7.5 but it can also tolerate mildly acidic to alkaline soils with a pH range of 5.0 to 7.8 (Hannaway et al., 1999). *L. multiflorum* competes severely with other plant species. Ryegrass, being an annual, has an advantage of rapid growth due to its short life cycle and is reported to survive at a pH of 8.4 which is far above the extreme conditions reported in literature, suggesting local evolutionary changes after introduction (Dawson et al., 2007) .

Venuto (2002) reported a mean seed mass of 2.6 mg for annual ryegrass diploids and 4.6 mg for tetraploid seeds. Germination rate in annual ryegrass ranged from 78.8% (Ribeye) to 98% in Jackson; 82.3% (Rustmaster) to 98.3% (Big Daddy); 77.8% (Rio) to 98.3% (Big Daddy) in 1997, 1998, 1999 respectively. Both seed weight and germination rate affect Pure Live Seed (PLS) m^{-2}. Larger seeds produce larger seedlings than smaller seeds do, but this difference is not apparent one month after sowing. Venuto et al. (2002) recommended that the seeding for forage be calculated based on PLS.

The test weight of annual ryegrass is normally about 309 kg m^{-3} and the approximate no. of seed/kg is 50,000. Seeding rate is 28 kg ha^{-1} when seeded alone and 22 kg ha^{-1} when sown in a mixture at a depth of 0 to 12.7 mm (Ball et al., 2002).

In annual ryegrass, dry matter yield is typically the key characteristic for improvement. Dry matter production is associated with photosynthesis and respiration rates. In cool season grasses, the highest photosynthetic rate occurs at 10 to 15 C and respiration rate increases exponentially with an increase in temperature. Among cool season grasses, annual ryegrass is susceptible to high temperature and is comparatively resistant to low temperature. Optimum temperature required for maximum photosynthesis is 10 to15 C (Murata and Iyama, 1962).

Forage Yield and Quality

Due to its high palatability, forage quality, seedling vigor, persistence under close grazing and dry matter yield, annual ryegrass is considered one of the most important cool season forage grasses. These attributes extend its utility as a pasture, hay, silage and cover crop. It is estimated that annual ryegrass is grown on 1.2 million ha in USA; 90% of that area is located in the Southeast (Balasko et al., 1995). As forage hay production is low and nearly impossible during winter, annual ryegrass is used primarily for grazing beef cattle, both in cow-calf as well as in stocker operations. Beef cattle producers need a continuous supply of high quality forage for cost-effective animal production, and annual ryegrass fulfills that need for winter and spring pastures. Its highest forage quality occurs during winter as compared to spring (Balasko et al., 1995).

Italian ryegrass attains full growth very quickly. When it is autumn-sown as is customary in the southeastern USA, 40% of the total forage yield occurs as early season (December-February) growth with remaining 60% as late season (March-May) growth. In one study, 30% of its total production occurred during April alone (Redfearn et al., 2002). This growth pattern makes ryegrass useful as a means of enhancing early production as compared to other grasses (Spedding and Diekmahns, 1971).

Annual ryegrass has high seedling vigor and makes fast growth, it produces abundant palatable forage, has an extensive root system, and the seed is relatively inexpensive. An important feature of this crop is its interference with other species in seed mixture or when it is present in an area to be seeded, which is due to fast growth rate or allelopathic effect on growth of other species. It is noted that growth of unwanted species (weeds) was lower in ryegrass than in tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh. = *Schedonorus arundinaceus* (Schreb.) Dumort.). Annual ryegrass is very competitive due to rapid increase in plant size, which dominates the microenvironment. It produces a greater mass of dense surface roots than harding grass (*Phalaris tuberosa* L.), wild oat (*Avena fatua* L.), soft brome (*Bromus mollis* L.) or Kentucky bluegrass (*Poa* *pratensis* L.). Dry matter yield of other species that were grown as test species with annual ryegrass was reduced significantly as compared to having been grown in an open area (McKell et al., 1969). Plants weighed less than half as much due to the effect of ryegrass. It has been demonstrated that remaining annual ryegrass litter had a significant influence on species composition in plots the following year. The greatest abundance occurred in conditions where residue remained from previous years, and secondly in plots from which residue was removed after plant maturity. The height attained by various species has a close relationship with growth of annual ryegrass because vegetation was shortest where annual ryegrass was most abundant (McKell et al., 1969).

Both forage quantity as well as forage quality is lacking in the southern USA during late autumn and early winter, often necessitating supplemental feeding. Availability of early ryegrass grazing would enhance the profitability of beef cattle operations. In 1997 Gulf and Marshall were compared in a grazing experiment on the basis of animal weight gain (Bransby et al., 1997). In South Alabama, weight gain was 77% more for Marshall than Gulf and 27 % more in North Alabama. Marshall performed better than Gulf in terms of both cold and grazing tolerance. Moreover management practices that increase early production could be beneficial for reducing supplemental feeding (Redfearn et al., 2005). Numerous cultivars are marketed in the southeastern US. Gulf is preferred in mildly cold regions, whereas Marshall is recommended in areas that may experience below freezing temperatures during the winter months (Redfearn et al., 2002).

Sod seeding of annual ryegrass into warm season grasses such as bermudagrass (*Cynodon dactylon* [L.] Pers.) results in efficient utilization of land and provides good quality forage for winter grazing. It has also been found that weight gain and apparent hay consumption by calves is more with winter annuals such as ryegrass, ryegrass + rye (*Secale cereale* L.) and wheat (*Triticum aestivum* L.) + ryegrass as compared to hay plus supplement. Therefore annual ryegrass alone or grown with other winter grasses provides winter grazing for fall weaned calves and reduces the consumption of hay and supplements and thus results in more weight gain in calves (Beck et al., 2007).

A study was conducted to learn the effect of cool season grasses on calf growth in which annual ryegrass was grown alone or with small grains. It was observed that the addition of annual ryegrass to small grains increased the number of animal grazing days per hectare and also increased body weight gain per hectare. By the end of spring grazing (May 7) annual ryegrass with small grain cereals resulted in an increase in forage dry matter by 17% from 1350 ± 52.3 kg ha^{-1} for small grains alone to 1582 ± 52.3 kg ha^{-1} with addition of ryegrass to small grains. Annual ryegrass makes most of its growth in spring, so it is helpful in extending the grazing season when grown with small grains, but it can be available throughout winter if winters are mild and wet (Beck et al., 2007).

Annual ryegrass nutritional quality decreases rapidly after heading (Table 1.01). Crude protein (CP) decreases with maturity. Neutral detergent fiber (NDF) increases with progress through the growing season, which occurs first in early maturing cultivars such as Gulf and Rustmaster.

It has been recommended that annual ryegrass should be harvested for hay or silage when 10 to 20% inflorescence has emerged because at this stage vitro dry matter digestibility (IVDMD) content will not be less than 770 g kg^{-1} DM. By using growth stage as an indicator to schedule harvest, dry matter yield and quality can be optimized regardless of maturity type and accumulation period. IVDMD and CP are negatively associated with length of the forage accumulation period. Water soluble concentrates increased with increase in maturity and hence increases ensiling properties (Callow et al., 2001). In winter, CP decreases and fiber content increases which is caused by senescence of leaves due to cooler weather. This period is then followed by an increase in CP and a decrease in fiber content because of spring regrowth in March and April (Beck et al., 2007).

Seed Yield and Production

With many field crops such as corn, seed production and agronomic production e.g. for dry matter, are often in the same geographic area. However, this is not the case with cool season forage crops. Annual ryegrass seed is mostly produced in the Pacific Northwest, primarily in Oregon and Washington, because of the conducive environmental conditions such as mild temperatures along with good rainfall in winter and spring and favorable dry summers for seed maturation and harvesting (Youngberg and Wheaton, 1979). However, the major agronomic utilization of annual ryegrass is in the southern USA. Cool season grasses such as annual ryegrass, orchardgrass, and tall fescue require cool and moist environmental conditions during initial growth, but warm dry conditions for seed maturation. A uniform climate results in high and stable seed yield. In particular, dry weather but with adequate residual soil moisture results in high quality seed crop.

The time gap between late summer seed harvest in the Pacific Northwest and autumn seeding in southern USA is relatively short. Weather conditions and transportation make it difficult to get new crop seed to southern farmers in autumn, especially for late maturing perennials. This tends to increase transportation costs for ryegrass seed (Jung et al., 1996).

Seed yield is a complex trait with low heritability (Burton and DeVane, 1953). There are few published studies on heritability of seed yield of cool season forages even though seed yield is a very important trait because it determines the economic viability of a superior cultivar. Stratton and Ohm (1989) conducted an experiment in Indiana and Oregon to correlate seed yield component traits with seed yield in orchardgrass. Yield per panicle ($r = 0.10$ to 0.67) and panicle number ($r = 0.46$ to 0.77) are indicators of seed yield but both are negatively and significantly correlated with each other. Seed yield per panicle, panicle number, and seed yield per plant were significantly higher in Oregon than Indiana indicating genotype × location interaction, which was attributed to longer growing days and favorable environmental conditions. Low phenotypic and genotypic correlation existed between locations for panicle number ($r = 0.06$ to 0.54), seed yield per panicle ($r = -0.08$ to 0.68), and seed yield per plant ($r = -0.19$ to 0.55). This indicates that seed yield data collected in Indiana had limited value in predicting seed yield in Oregon. So it is critical for plant breeders to know whether selection made for seed yield in the eastern USA actually improves seed yield in Oregon (Stratton and Ohm, 1989). The practical conclusion is that one cannot select for seed yield outside the target area of production. Similarly, the lack of progress in increasing yield of annual ryegrass documented by (Venuto et al., 2002) may also be the result of selection for forage yield outside the target area of agronomic production as many newly marketed cultivars were selected in Oregon rather than in the southern USA.

Breeding Objectives

In recent years scientists and livestock producers have put more focus on maximizing animal intake through grazing and minimizing the feeding of preserved forage (Ball et al., 2008). Keeping animals grazing is desirable for several reasons, but especially due to lower feed cost compared to feeding hay, silage, or other stored feeds. Whether grazing is possible depends obviously on the weather - severe summer droughts may necessitate supplemental feeding- but also depends on the species and geographic region where forages are grown. Warm season grasses predominate in the lower South (LA, MS, AL, GA, FL, SC), whereas cool season perennial grasses become more important in the transition zone (AR, TN, KY, MO, NC, VA, WV, and the southern counties of IL, IN, and OH), although bermudagrass, e.g., yields quite well in some areas in some of these states. The yield distribution of warm season perennials peaks in mid-summer (Fig. 1.02).

Cool season perennials such as tall fescue tend to have a bimodal yield distribution with the main peak occurring at the conclusion of reproductive growth in the spring and a secondary peak occurring in mid autumn. The distribution of dry matter yield of annual ryegrass is unimodal but peaks somewhat earlier than tall fescue. However, while most tall fescue cultivars, with the exception of Mediterranean types such as AU Triumph, are basically winter dormant, ceasing growth in late autumn, annual ryegrass continues to grow depending on temperature and moisture availability. Due to less availability of winter annual and perennial forages during late autumn and early winters, animals are usually fed hay in order to meet their nutritional requirements, which in turn increase management costs.

It would be highly beneficial for livestock producers to have a solid live forage base during the cooler months of the year. There are agronomic and plant breeding solutions to this problem, the former are quicker to implement and might only involve minor adjustments. One such approach would be to seed earlier than commonly done. Producers tend to wait until autumn rains arrive before they start seeding annual ryegrass. Annual ryegrass is tolerant of late summer (early September) seeding; seeding even before the rain arrives may prove to be beneficial. Supplemental irrigation (approx 50 mm for the first two weeks after seeding) enhances rapid stand establishment in the absence of rain and annual ryegrass can then respond to autumn rain with rapid dry matter accumulation of 1000 kg ha^{-1} or more by early November (van Santen, 2008, personal communication). This approach could be combined with establishing annual ryegrass in warm season perennials such as bermudagrass (*Cynodon dactylon* L.) and bahiagrass (*Paspalum notatum* Flügge), which can only be overseeded successfully during early autumn after they begin to become dormant. If some of the summer forage is stockpiled, a multi-tiered approach could lead to a stable forage base satisfying most of the animal requirements during autumn and winter.

Annual ryegrass is generally one of the most productive winter annuals and produces maximum DM growth during late winter and spring. The average performance of annual ryegrass over three years (2006 - 2008), was estimated at a fewer Alabama locations using 20 to 30 cultivars. Dry matter yield varied under different growing conditions. At Crossville, AL the first cut was done in March, and peak yield occurred in late spring (May), whereas for Tallassee, Fairhope and Headland the first harvest for dry matter was during December and maximum production was attained in March. In general, cultivars produce dry matter from fall to late spring, with maximum seasonal production during early spring (March and early April) followed by fall (through February) and late spring after April 20 (Glass and van Santen, 2008a). Small grains attain maximum forage yield during early spring (March and early April) with varying production during autumn (through December), winter (January and February) and late spring (after April 20) under different conditions (Glass and van Santen, 2008b). Annual ryegrass along with small grains can extend grazing days as compared to small grains alone. Thus annual ryegrass can be made available during early winter through agronomic means.

Another approach would be to improve the growth rate of annual ryegrass during the cooler months of the year through genetic means. The objective of such a plant breeding approach would not necessarily be the improvement of total seasonal yield but to shift some of the yield distribution to the cooler months. This approach would take longer because it involves an increase of desirable alleles for early DM production, a feat that can be accomplished only over several generations of recurrent selection. It takes at least 10 years to develop and release a new cultivar.

Breeding Methods

The breeding method used for a given crop depends on several factors. Among these factors is the desired cultivar type for commercialization, which in turn, depends on the reproductive system and reproductive value of the crop. For vegetatively propagated crops such as blueberry (*Vaccinium corymbosum* L.) a pick-the-winner type approach is used, where parental clones are hybridized and the best progeny is then vegetatively propagated. A similar approach works for vegetatively propagated turf hybrid bermudagrass (*C. dactylon* x *C. transvaalensis* Burtt Davy), where the breeding approach involves species hybridization. Maize is primarily marketed as F-1 hybrids and the breeding methods involve hybridization followed by the extraction of inbred lines. These lines are developed by controlled selfing with selection or backcrossing. In self pollinating crops, including small grains and pulses, pure lines are released as cultivars where heterozygosity decreases by 50% with each generation and hence a practical stage of homozygosity can be attained in 5 to 7 generations to develop a pure line. In cross-pollinated crops, e.g. red clover (*Trifolium pratense* L.) and perennial ryegrass (*Lolium perenne* L.), where heterozygosity is exploited, populations are improved by increasing the frequency of desired alleles. For seed propagated cross-pollinated forages, where controlled pollination is difficult due to perfect flowers and self fertilization is restricted due to self incompatibility, synthetic cultivars are produced by seed mixtures of inbred lines, strains or hybrids and propagated for a few generations by open pollination.

The sexual reproductive system places restrictions on the breeding methods. Some species are almost exclusively autogamous because of floral morphology. An example is soybean (*Glycine max* (L.) Merr.), where cleistogamous flowers enforce self-pollination. Some flowers open after anthesis which increases chances of self-fertilization such as in wheat and barley. This mechanism is called chasmogamy in which pollen shedding occurs before flower opening, which increases chances of self-pollination but out crossing can occur after flower opening. In *Monnochoria vaginalis* (Burm. f.) C. Presl ex Kunth, emasculation of chasmogamous flowers just after flower opening has resulted in seed set, which indicated that chasmogamous flowers have self pollination mode but out crossing can also occur. Crossing behavior can vary with environmental conditions such as light intensity (Imaizumi et al., 2008). Intermediate are monoecius species such as corn that have separate pistillate and staminate flowers on the same individual. In these species breeding methods based on self- as well as cross-pollination may be used. An enforced system of allogamy is present in dioecious species such as hop (*Humulus lupulus* L.) that have a sex determination mechanism resulting in female and male plants. Outcrossing in practice is enforced by male sterility, where plants fail to produce functional anthers or viable pollens but have normal ovaries in hermaphrodite flowers. This restricts self pollination and thus results in cross pollination. Examples are barley, corn, cotton, and soybean. It has been used commercially in maize and pearl millet (*Pennisetum glaucum* (L.) R. Br.) to produce hybrid seed. Maturation of anthers and stigmas at different times in the same or different flowers of a plant, called dichogamy, results in cross-pollinations. Protogyny in pearl millet (*Pennisetum glaucum* (L.) R. Br.) and protandry in maize (*Zea mays* L.)are examples of dichogamy. Physiological hindrances such as self incompatibility or self sterility, in which viable pollens are not capable of fertilizing with viable stigmas results in cross pollination. This mechanism occurs in grasses and crucifers, where it is utilized for crop improvement.

Finally, the influence that the environment has over the expression of a trait, i.e., heritability, has a strong influence over the breeding methods utilized. Heritability has broad implications in plant breeding as it can be applied for selection within randomly mated cross pollinated populations, self fertilized lines, or to test cross progenies in hybrid crops and clones. Hanson (1963) defined heritability as the fraction of selection differential expected to be gained when selection is done on a defined selection unit (Hanson, 1963). The effectiveness of a breeding method also depends upon the heritability of a trait. Traits with high heritability can be improved easily with less evaluation than traits with low heritability. For traits that have low heritability, other approaches such as progeny testing should be done in order to know the exact genetic potential of the parents.

Phenotypic and genotypic variance of the trait should be known, moreover genotypic and phenotypic correlations among traits are necessary for indirect selection. Phenotypic correlation consists of genotypic and environmental correlation. Phenotypic correlation can be greater or lesser than genotypic correlation due to environmental factors because environmental correlation can effect traits in the same direction or in opposite directions. Therefore, the difference between phenotypic and genotypic correlation reveals the environmental correlation. If genotypic and phenotypic correlations are significantly similar, then selection can be done on the basis of phenotypic correlation only. If phenotypic and genotypic correlations are almost the same, environmental correlation is absent. Heritability estimates of different populations can help a plant breeder in selecting appropriate populations to be used as the base population (Goodman, 1965). After generating a base population, several questions should be considered by a plant breeder, for example how much genetic variation is present in the material, how extensive testing will be required over years, locations etc., what breeding method will be used for maximum improvement, and what type of cultivar should be produced (hybrid, pure line, synthetic) (Dudley and Moll, 1969).

Comparing heritabilities of different family structures originating from the same base population facilitates choosing a family structure that maximizes genetic gains over a specific time period (Burton and Carver, 1993). Heritability varies among traits within one population, so traits with high heritability and a strong genetic correlation with trait of interest can be selected so as to apply more effective indirect selection method than direct selection (Banziger and Lafitte, 1997).

Recurrent Selection

All recurrent methods share three phases: evaluation, selection and recombination. Creation and evaluation of base population is also important phase of recurrent selection but it depends on evolution of crop and pollination mechanism. For example this step is more critical in highly domesticated and selfed crops where selection of diverse cultivars with different origins is important for population improvement. Recurrent methods can be broadly subdivided into two categories. Phenotypic recurrent selection includes improvement of quantitative traits through evaluation and selection of plants, then intermating selected plants which act as base for the next cycle. Whereas, in the genotypic method, selection of plants is done on the basis of their progeny performance and then selected parents and/or progeny are intermated to produce seed for the following generation.

Under half sib genotypic recurrent selection, desired plants are harvested individually to allow growing of their progenies in the second season in isolation. The population is reconstituted in the third season by compositing seed from superior progenies or from the parents of superior progenies, hence completing one cycle of selection in two years. Full sib selection includes pairwise crosses among selected plants in the first season. In the second season, progenies are grown in isolation from selected pairs for evaluation and the population is again regenerated by compositing the remnant seed of superior progenies. The reciprocal recurrent selection includes improvement of the two populations simultaneously by selfing and crossing of populations in the first season and replicated tests of half sib families in the second season. In the last season, remnant seed of selected families is intercrossed to generate a population for next generation. This method cannot be applied for self-incompatible crops such as ryegrass.

Maize (*Zea mays* L.), an allogamous and monoecious species, has been used as a model crop for various breeding methods including recurrent selection. Male and female flowers develop on same plant but are physically separated. Anthers mature earlier and disperse pollen through wind currents whereas silk emergence from the cob occurs 1 to 3 days after pollen dehiscence. Under natural conditions protandry results in cross-pollination with very little selfing. Pollen shedding may vary from a few days to more than a week depending upon environmental conditions such as temperature, air movement, humidity etc. Maize can be just as easily selfed as it can be hybridized, hence various methods can be used for breeding purposes due to this flexibility in mating.

Phenotypic recurrent selection is easy and quick to conduct as this method needs one year per cycle for annual crops and improvement is continuous. In maize, mass selection or phenotypic selection gained 0.029 Mg ha^{-1} per cycle or per year at a cost of $ 350 per unit of gain (Table 1.02) (Weyhrich et al., 1998). Half and full sib selections resulted in higher gains per cycle but gains per year were similar to mass selection because completion of one cycle takes at least two years compared to a single year for mass selection. In addition, these two methods require a large amount of money because of the progeny tests involved, and hence give lower returns compared to mass selection. Therefore, a larger number of plants can be evaluated and intermated in mass selection than with the other two methods. There is no doubt that modified ear-to-row gives the highest returns with less time among all seven methods but as it involves selfing, this method cannot be applied to annual ryegrass because of the strong self incompatibility. Therefore forages are mostly improved through mass selection method due to a shorter time period being required and because results are economically more beneficial. Further efficiency of phenotypic selection can be improved with gridding and increased selection intensity.

Recurrent selection increases the frequency of desirable alleles while maintaining genetic variation, i.e., additive variance in population, to facilitate long-term selection as shown in Fig 1.03. Frequency of favorable alleles increases in the improved population over the base population. Progress per cycle is evaluated by increases in the generation mean. Sometimes responses are not evident from cycle to cycle and become very erratic. Gradual improvement is realized from long-term selection. Large sized populations prevent loss of alleles that occur with very low frequency. Total variance consists of additive, dominance and epistatic variances. Phenotypic selection utilizes only additive variance (the fixed part of variance) and epistatic variance with additive component (Hallauer and Miranda, 1981).

With recurrent selection in maize for grain yield, gene frequency for red cob (PI-*wr*) increased linearly with selection cycles (Frascaroli and Landi, 1998). With consequent cycles, allelic frequency increased while maintaining standard error. Selection and recombination of superior progenies or families at each selection cycle increases the frequency of favorable alleles, which provides the basis for further improvement.

Response to selection depends upon selection intensity and heritability (h^2) of a trait. The general form of expected response to selection is

$$\Delta G = \frac{k\,\sigma_G^2}{\sigma_P}$$

Expected progress from phenotypic selection with no pollen control is assessed by the following formula;

$$\Delta G = \frac{k(\frac{1}{2})\sigma_A^2}{\sqrt{(\sigma_A^2 + \sigma_D^2 + \sigma^2 + \sigma_{we}^2)}}$$

where the phenotypic variance $\sigma^2_P = \sigma^2_A + \sigma^2_D + \sigma^2 + \sigma^2_{we}$, $\sigma^2_A + \sigma^2_D$ = genetic variance, σ^2 = plot to plot environmental variance, and σ^2_{we} = within plot environmental variance. The relationship between parent – offspring is determined as (1/2) σ^2_A.

Examples of Recurrent Selection in Forage Species

Recurrent selection is considered as the best method to enhance germplasm and to develop cultivars. This selection requires genetic variation, the sexual mode of reproduction, and seed producing cross-pollinated plants. It is also applicable to self pollinating crops, but as extensive cross pollination is required, which poses a challenge in autogamous crops, and moreover sufficient seed may not be available after crossing. Because forage crops have high variability, less uniformity is usually required, recurrent selection is better suited to forage crops and plant yield is a more favorable character than seed yield. This method enhances traits without inducing undesirable characters. A prime example is the development of the ryegrass cultivar Marshall, regarded as one of the best cultivars in the southern USA (Bransby et al., 1997).

The success of recurrent selection depends upon the genetic makeup of the base population. Parents selected to generate the base population should be high performing. To create a base population, a greater number of random mating cycles increase the recombination between genes, and the population goes into linkage disequilibrium. Gain from selection in breeding methods in cross-pollinated crops such as switchgrass (*Panicum virgatum* L.) depends upon the genetic variability for the desired trait present in the base population (Bos, 1983).

Phenotypic selection along with use of the grid method tends to reduce environmental variations in which plants are arranged in *n* blocks with *m* plants per block. A fixed number of plants are selected from each grid to keep the selection intensity constant across blocks. This restriction on phenotypic selection was developed by

Gardner (1960), who divided a field into small uniform plots to minimize genotype-environment effects and allow maximum expression of genotype to select the highest yielding plants in corn. This method increases the frequency of desired genes in a population. (Burton, 1992).

Recurrent phenotypic selection was initiated in 1985 to improve cereal rye (*Secale cereale* L.) forage yield. This method was modified after Burton's selection in bahiagrass by restricted recurrent phenotypic selection (RRPS) in 1982. In rye breeding, after applying four cycles RRPS) an average increase of 6 to 7 % per cycle was reported in four populations of cycle 1 through cycle 4 in spaced plant yield and 0 to 3 % in seeded plot yield. Plants were selected on a visual basis, which have shown high positive correlation with fresh weight such as 0.90, 0.84, 0.82, and 0.89 for various harvests of rye during RRPS (Bruckner et al., 1991). Similarly in perennial ryegrass (*Lolium perenne* L.), a genetic correlation of 0.84 was found between dry matter and green matter yield (Conaghan et al., 2008). So these correlations indicate that visual and green matter selections are effective criteria for phenotypic selection in spaced plants for high forage yield. This selection is considered efficient in terms of time, labor, and space (Bruckner et al., 1991). Burton, in 1982, reported forage yield increase of 16.4% per cycle in space planting for first eight cycles of RRPS and without any significant decrease in genetic variability in bahiagrass where selection was done visually (Burton, 1982).

It has been reported in rye that 15 cm plant spacing shows negative yield response whereas there is a positive relation in 90 cm spacing. Competition among plants increases with a decrease in spacing and results in low yield. Thus, yield and competing ability are negatively correlated. High yield response in spaced plants is due to genetic differentiation between high and low yield genotypes (Kyriakou and Fasoulas, 1985).

In Pensacola bahiagrass (*Paspalum notatum* Flügge var. *saurae* Parodi), , it was suggested that the rate of progress in yield increase can be doubled by using one year per cycle instead of two years, and moreover by reducing plant selection per grid from 5 to 4 or 3. By using 20% selection intensity in four cycles of RRPS, forage yield increased by 16 to 19 %. Burton used some restrictions to improve the efficacy of RRPS. This approach doubles the rate of progress by having control over both the parents as selection occurs before anthesis (Burton, 1974).

Marshall and Wilkins studied the effectiveness of phenotypic recurrent method in increasing seed yield in perennial ryegrass. After two cycles of selection, seed yield per plant in the selected population was significantly higher than the unselected population due to high seed set, a greater number of reproductive tillers and a higher number of seeds per tiller. Therefore, phenotypic selection resulted in increased seed yield by altering seed yield components (Marshall and Wilkins, 2003).

In Pensacola bahiagrass, yield increased consistently for eight cycles, and a 16.4% improvement occurred per cycle of restricted recurrent phenotypic selection (RRPS). As compared to the commercial cultivar, cycle 6 yielded 91% more in spaced plants and 84% more in seeded plots. Improved RRPS allows one cycle per year, which reduces the evaluation time period to half and hence increases efficiency by four times as compared to the conventional method in bahiagrass yield. Linear relationship of yield increase through 8 cycles without any loss in genetic variation is indicated by coefficient of variation values that foretell further possible improvement (Burton, 1974).

In Pensacola bahiagrass, cycle 16 and cycle 9 of RRPS and commercial Pensacola bahiagrass were compared for morphological traits. Cycle 16 had the highest mean values for culm number per plant, plant height, leaf length, mean leaf weight, culm weight and whole plant weight. So it was inferred that RRPS resulted in an increase of these morphological traits except for plant diameter which decreased with cycles. So the increase in forage yield can be attributed to increases in measure of morphological traits. Plant weight, as measured by multiple regression, is measured by leaf length and number of culms (Werner and Burton, 1991).

In annual ryegrass, dry matter is a key trait for improvement. In order to assess dry matter yield, many conventional cutting methods are used such as estimation on the basis of dry weight, which are time consuming and laborious. Therefore, dry matter estimation through related traits, called indirect selection, has an important influence on ryegrass breeding. Indirect selection is the selection of primary trait through selection of secondary traits. For efficient indirect selection, a secondary trait must be highly correlated to the primary trait and show high heritability (Gallais, 1983). In annual ryegrass, it has been reported that a strong correlation exists between dry matter and fresh matter, dry matter and plant vigor, and fresh matter and vigor with values of 0.96, 0.84, and 0.90 respectively, where plant vigor is measured in terms of plant height, number of tillers, and number and size of leaves (Mittelmann et al., 2006). Dry matter yield is taken as a direct response and green matter and vigor are considered as indirect responses. For green matter selection, plants are evaluated and selected on the basis of fresh weight, and for vigor response, plants would be evaluated and selected in the field on the basis of morphological characters without consuming much time and labor. Therefore because of easy evaluation and high association of green matter and vigor with dry matter yield, these can be used as indirect selection criteria in annual ryegrass and its use is more appropriate in mass selection or preliminary selection trials (Mittelmann et al., 2006). Similarly in perennial ryegrass, fresh matter was considered an efficient method to use to select for high dry matter yield (Conaghan et al., 2008).

Essentially, a review of literature emphasizes that in the southeastern USA, winter annual and perennial grasses have high spring production but relatively less early winter production. Consequently, the first objective of this research is to increase early winter production in annual ryegrass. Annual ryegrass was selected for forage improvement because of its vigorous growth, early establishment, high palatability and excellent dry matter yield. Moreover, its spring peak production is earlier than winter perennials. Phenotypic recurrent selection method can be used to improve winter production in annual ryegrass because this crop has self-incompatibility which results in cross pollination. It improves the mean value of the trait with constant genetic variability. Phenotypic recurrent selection with grid restrictions was used to reduce heterogeneity in blocks, and plants are selected on the basis of direct (dry matter yield) and indirect responses (green matter, visual observations, and percent dry matter).

The second objective was to study the correlated responses such as heading date, plant type, seed yield and disease frequency in the base population, cycle-1 dry matter population and cycle-2 dry matter population to measure the response to selection. After two cycles of phenotypic recurrent selection, cycle-2 populations along with cycle-1, the base population and check cultivars were evaluated at five locations in Alabama. The objective was to measure the seasonal yield distribution and to compare C-2 dry matter population with the base population and check cultivars. The dry matter yield of C-2 dry matter population (direct response) was also compared with dry matter yield of indirect responses.

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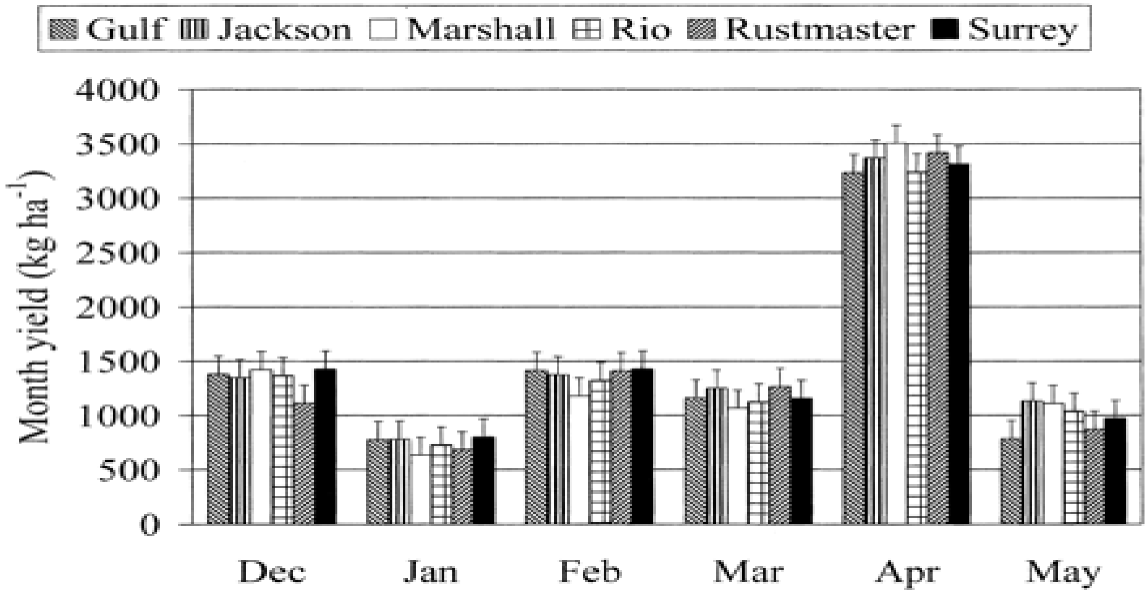

Figure 1.01: Yield distribution for six annual ryegrass cultivars in different months across four different locations. Yield decreased in January and increased drastically in April. During early months, Gulf has more production however Marshall has higher production during late months. Source: Redfearn et al. (2002).

Table 1.01: Mean crude protein (CP), neutral detergent fiber (NDF), in vitro true digestibility (IVTD), and digestible NDF (DNDF) of annual ryegrass cultivars. Source: Redfearn et al. (2002).

Cultivar	December	January	February	March	April	May
	------------------------------------- $g\ kg^{-1}$ --					
			Crude Protein (CP)			
Gulf	251	234	262	190	172	129
Jackson	255	229	260	183	175	134
Marshall	245	232	262	181	178	131
Rio	247	238	265	185	184	134
Rustmaster	244	244	265	191	170	129
Surrey	250	241	265	187	183	130
LSD (0.05)	NS	NS	NS	NS	3	NS
			Neutral Detergent Fiber (NDF)			
Gulf	406	422	416	430	547	606
Jackson	389	400	407	429	520	566
Marshall	371	382	395	400	510	557
Rio	390	401	403	411	505	574
Rustmaster	393	404	420	429	540	605
Surrey	391	399	408	417	513	584
LSD (0.05)	NS	6	5	12	8	14
			In Vitro True Digestibility (IVTD)			
Gulf	840	825	847	824	749	688
Jackson	849	836	848	825	770	711
Marshall	846	843	853	835	777	722
Rio	841	833	850	833	779	710
Rustmaster	837	833	848	828	756	685
Surrey	841	836	851	830	769	702
LSD (0.05)	2	4	NS	NS	4	5
			Digestible Neutral Detergent Fiber (DNDF)			
Gulf	598	583	629	591	543	489
Jackson	590	588	624	596	560	493
Marshall	574	588	623	588	565	504
Rio	582	582	625	596	562	497
Rustmaster	578	584	636	599	550	482
Surrey	585	587	632	592	551	493
LSD (0.05)	NS	NS	NS	NS	10	4

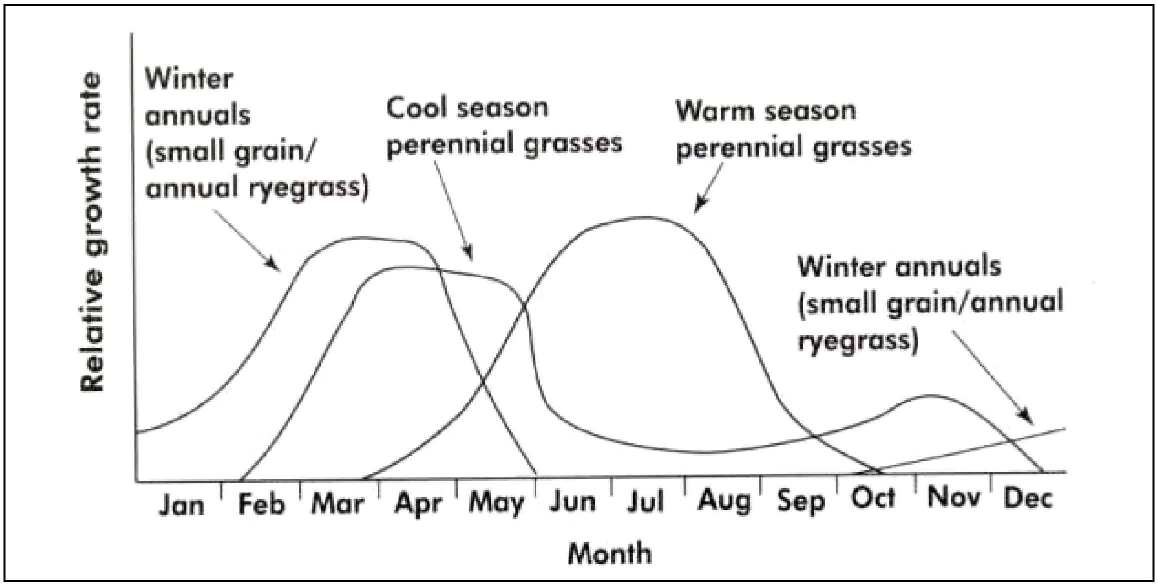

Figure 1.02: Seasonal dry matter yield distribution in cool and warm season grasses. Source: Ball et al., (2002).

Table 1.02: Comparison of six recurrent selection methods to improve grain yield in the BS11 maize population. Source: Weyhrich et al., (1998).

Selection method	Gain per cycle	Gain $year^{-1}$	Ave. cost $cycle^{-1}$	Ave. cost $year^{-1}$†	Cost $unit^{-1}$ of gain‡	Time to achieve one unit of gain§	Return on investment¶
	$Mg\ ha^{-1}$	$Mg\ ha^{-1}$	$\$Cycle^{-1}$	$\$Yr.^{-1}$	$#	Yr.	$Mg\ ha^{-1}\ \$^{-1} \times 10^5$
Full-sib	0.067	0.033	6 700	3 350	100 250	30	1.00
Half-sib	0.075	0.025	14 300	4 767	190 058	40	0.53
Mass	0.029	0.029	350	350	12 123	35	8.25
Modified ear-to-row	0.172	0.086	6 650	3 325	38 721	12	2.58
Reciprocal full-sib	0.124	0.062	12 100	6 050	97 213	16	1.03
S_1-progeny	0.091	0.046	7 300	3 650	79 888	22	1.25
S_1-progeny	0.212	0.071	10 300	3 433	48 530	14	2.06

† Calculated by taking cost $cycle^{-1}$ divided by the number of years required to complete one cycle.
‡ One unit of Gain is equal to a one $Mg\ ha^{-1}$ increase in grain yield. Calculated as the cost divided by the gain $cycle^{-1}$.
§ Calculated as the inverse of gain $cycle^{-1}$ multiplied by the number of years required per cycle.
¶ Calculated by taking gain $cycle^{-1}$ divided by the total cost $cycle^{-1}$.
# All calculations were made assuming a cost of $10 per nursery row, $15 per winter nursery row, $10 per yield trial plot, and a cost of an average size isolation of $350.

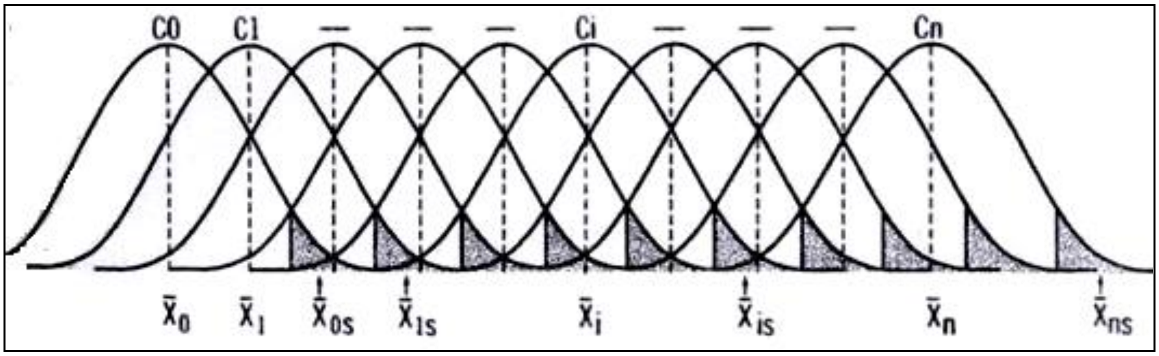

Figure 1.03: Mean increases with each generation while maintaining genetic variation. Source: Burton (1992).

II. PHENOTYPIC RECURRENT SELECTION FOR WINTER PRODUCTIVITY IN ANNUAL RYEGRASS (LOLIUM MULTIFLORUM LAM.) - SELECTION PROCEDURES AND CORRELATED RESPONSES

Abstract

Annual ryegrass is a major cool season forage crop in the southeastern USA that has a potential growing season extending from late summer to late spring. Due to less availability of cool season annuals and perennials during early autumn and winter months, animals are usually provided stored forages at these times. Annual ryegrass has high production during spring months but is lacking in late autumn and winter production. Thus, the primary objective of this breeding project was to shift the yield distribution of annual ryegrass more towards the winter months in order to make more forage available for grazing at this time, thus reducing the dependence of livestock on stored forages and grains. The base population was created from six top performing cultivars. Plants were polycrossed and bulk harvested for two generations to create a random mating base population (C_0). Phenotypic recurrent selection method was used as this crop is cross pollinated due to self incompatibility. Approximately 1200 plants were space-planted in blocks of 25 plants each during 2005/06 and 2006/07. For the first cycle, selection was done on the basis of dry matter to create C_1 followed by selection based on visual estimation of yield, green matter, dry matter percent and random selection along with dry matter in cycle 2 to study the effect of indirect selection criteria. One plant per

block was selected after 750 GDD for each selection criterion with 4 % selection intensity. Selected plants were recombined in isolated nurseries to generate seed for the next cycle. Therefore, along with the main objective of increasing early winter production, other objectives were to select alternative methods for plant selection such as on visual estimation of yield or green weight basis.

Seed from C_2 was used to establish replicated space planted seed increase nurseries (RCBD, r = 4) to multiply seed for a multi-location evaluation. Correlated responses such as erectness of plants, heading date, disease incidence and seed yield were collected during the reproductive phase. After two cycles of phenotypic recurrent selection, visual and green matter have shown high correlation with dry matter. All correlated responses except disease frequency have exhibited significant change with selection for dry matter production ($P < 0.0001$). Indirect selection was effective in improving visual, green matter and percent dry matter populations of C_2.

Introduction

Annual ryegrass, also known as Italian ryegrass, is a cool season bunchgrass, native to Southern Europe (Jung et al., 1996). It is a diploid species with 2n=2x=14 chromosomes and belongs to the family Poaceae. It is utilized as pasture, hay, silage and cover crop due to its high palatability, high forage quality, seedling vigor and persistence under close grazing. Annual ryegrass is grown on 9.3 million ha in USA, of which 90% area is constituted by southeastern states (Balasko et al., 1995) where it is utilized as a forage crop. However, seed production of annual ryegrass occurs in Oregon and

Washington (Youngberg and Wheaton, 1979). Selection of annual ryegrass outside its agronomic utilization area resulted has greatly limited genetic improvement during the last 20 years. To obtain good results, forage traits should be selected in their respective agronomic areas in order to increase forage production (Casler et al., 2003).

Due to the mild temperate to subtropical climate in the southeastern USA, the growing season of annual ryegrass is from late summer to spring. In this area, autumn grown ryegrass produces about 40% of its growth in winter months (December-February) and the remaining 60% in late season (March-May). In Louisiana approximately 30% of the total production occurs in April alone (Redfearn et al., 2002). Annual ryegrass attains a high growth rate during the spring months while less during cooler months. Due to limited availability of standing forages during these cooler months, cattle are fed stored forages and grains to meet their nutritional requirements which in turn increases management costs (Ball et al., 2002). Therefore research was conducted to increase early winter dry matter production.

It is easy to conduct recurrent selection in annual ryegrass as it is a cross pollinated crop due to self-incompatibility. Phenotypic recurrent selection includes evaluation, selection, and genetic recombination of plants. Selection of superior genotypes increases the frequency of desirable alleles in the improved population over the base population while maintaining genetic variance constant for long term selection. High dry matter yielding cultivars are developed through recurrent selection methods as this trait has low heritability value. This method has increased forage yield in Pensacola bahiagrass (*Paspalum notatum* Flügge) (Burton, 1974; Burton, 1982), rye (*Secale cereale*

L.) (Bruckner et al., 1991), maize (*Zea maize* L.) (Dudley and Lambert, 2004), and seed yield in perennial ryegrass (*Lolium perenne* L.) (Marshall and Wilkins, 2003).

Correlation among traits becomes important because provides knowledge of the effect of one trait on another. It can be helpful in determining and selecting highly heritable traits that can be correlated with yield. Therefore the objective of this project was to study correlated responses where selection was done on dry matter basis as a direct response. Correlated responses such as heading date, seed yield, plant type and disease frequency were collected.

Materials and Methods

Base population

Fifty plants selected from each of the five highly ranked cultivars in Alabama annual ryegrass performance trials were transplanted to a polycross nursery in autumn 2003. Gulf was also included because it has been a longstanding check cultivar in Southeastern trials that has not been outyielded consistently by any other entry (Redfearn et al., 2005). No selection was practiced and seed was bulk- harvested in June 2004. A 300-plant polycross nursery was established in autumn 2004 using seed from the first round of intermating. Again, no selection was practiced and seed again was harvested in bulk in June 2005. Two generations of random mating thus created the base population (C_0) for this selection experiment.

Selection procedures

Beginning with the C_0 population in August 2005, seedlings were established in conetainers (Stuewe & Sons, Inc., Tangent, Oregon, USA) filled with 1:1 mixture of PRO mix peat-based growing medium : sand. After emergence, approximately two weeks after seeding, seedlings were thinned to a single plant per conetainer. A minimum of 1075 plants per cycle were transplanted on 90-cm centers in a spaced plant nursery in early November at the Plant Breeding Unit (PBU) of the E. V. Smith Research Center, Tallassee Alabama. The trial area was prepared 3-4 weeks before transplanting and treated with a standard rate glyphosate 2-days prior to transplanting in order to create a stale seedbed. The number of rows and columns were arranged in multiples of 5 such that the entire field could be gridded in to 5 x 5 plant blocks plus a border surrounding the entire trial. Plants were left to grow for 750 GDD post transplanting corresponding to mid January to early February for the four cycles of phenotypic selection thus far completed.

After 750 GDD, a 2-person team visually identified and marked the best-yielding plant in each 25-plant block. Afterwards, every plant was harvested at a uniform height of 5 cm by collecting all tillers, including the extremely prostrate ones, and cutting them along a wooden block that served as a height gauge. Plant material was weighed to determine green matter yield, dried at 60 C for 3 days, and weighed again for dry matter yield determination. The ratio of dry matter to green matter yield (dry matter percentage) was also used as an evaluation criterion.

For cycle 1 selection was based on dry matter yield. Beginning in C2, selections were also made based on the following criteria: visual selection, green matter, dry matter percentage, and random, the latter being a single plant selected at random from each 25-

plant block to check genetic drift. The dry matter yield population was the only one used for recombination to generate seed for the next cycle; other selection criteria were being evaluated as indirect responses for a single cycle. The selection intensity was 4% as a single plant from a 25-plant block was selected. Based on a minimum of 1075 plants per cycle, at least 43 plants were recombined for the next cycle. Beginning with C_3, we arranged the selection nursery in such a way as to allow also for selection within a 6 x 6 plant block, resulting in a selection intensity of 2.8%. This was done only for dry matter yield. Thus from the C_2 selection, any criteria other than dry matter yield may also be viewed as an example of tandem selection, where selection was practiced for dry matter yield in the previous cycle followed by visual, green matter, dry matter percentage, or no selection (random mating only) in the current cycle. The randomly selected population may viewed in terms of the effect of one generation of random mating following selection.

Seed yield nurseries

For seed production, five C_2 populations along with the base population were seeded in conetainers in the greenhouse in late August 2007 using the same procedure as for the selection nursery. Replicated seed increase nurseries (RCB, r = 4) were then established at PBU in early November 2007 from this greenhouse grown material. This enabled us to investigate correlated responses for seed yield, heading date, plant growth type, and disease reaction. Each plot consisted of approximately 200 plants on 90-cm centers arranged in an equal number of rows and columns to minimize the average distance between plants and promote random mating. Plots were surrounded on all sides by a 10-m border of cereal rye (*Secale cerale* L.) cv. Wren's Abruzzi to prevent pollen flow. This rye cultivar is at least 60 cm taller than annual ryegrass at time of anthesis and is very effective in preventing pollen flow.

Individual plant notes were taken during reproductive development by visiting the plots every three days. The heading date for a given plant was the day when at least five spikes had emerged 75% from the boot. Plant type was scored at that stage on a scale of 1-5, where one represented a prostrate plant with a tiller angle less than 30˚ and five an erect plant with tiller angle greater that 75˚.

In late May 2008 when the rachis below the spikes had turned from green to brown, seed was harvested separately from random plants within each plot for another study investigating genetic variation in these populations. The remainder of each plot was bulk harvested, dried in a shed and threshed with a stationary thresher. Seed was then cleaned with an Airblast Cleaner (ALMACO, Nevada, IA) with a common airflow setting for all plots. This seed will be used to evaluate progress from selection in solid seeded plots.

Results and Discussion

Selection procedures

As indicated in the Materials and Methods section, the main selection criterion was individual plant dry matter yield, for which the best plant in each 25-plant block was selected for recombination to generate the population for the next cycle.

Along with selection on a dry matter yield basis, plants were also selected based on indirect responses such as green matter (= fresh weight) and visual scoring for yield to evaluate alternative and potentially more cost-effective methods. For visual selection a 2-person team independently selected the best-yielding plant and arrived at a consensus when the initial assessment differed. Due to the lack of adequate resources it was impossible to carry three populations forward, so indirect selection was conducted for each cycle separately.

For the fresh weight selection, the weight before dry-down was the criterion used. The top-ranked plant based on fresh weight would have also been selected as the top-ranked plant based on dry matter yield in at least 70% of all cases (Fig. 2.01, right columns) and in close to 100% of the cases, the top three for dry matter yield. Visual selection was not nearly as effective as selection based on fresh weight, although the top-ranked entry based on visual selection included ranks 1- 4 for dry matter yield in over 95% of the cases (Fig. 2.01, left columns). A learning process can be surmised from the differences in cycles 1- 4 but full coincidence has not yet been reached. Similar results were found by Mittelmann et al.(2006) in annual ryegrass where strong correlation was found between fresh weight and dry matter; dry matter and vigor; and fresh matter and vigor with values of 0.96, 0.84 and 0.90 respectively (P = 0.01). In rye (*Secale cereale* L.), during phenotypic selection on spaced planting, plants selected on the basis of visual selection showed a high positive correlation with fresh weight ranging from 0.82 to 0.92 (Bruckner et al., 1991).

Correlated responses observed in seed yield nurseries in 2008

Correlated responses are the changes in non-selected traits that occur when selection is practiced for the target trait. Correlated traits estimated in forages have shown that highly heritable characters can be selected along with a complex trait such as yield. As indicated in the Materials and Methods section, we took notes on certain growth parameters in the replicated seed increase nurseries to evaluate the traits that might have also changed when we selected for increased winter growth, which also indicates the presence of genetic variation in populations for these traits.

The C_2 random population is representing C_1 dry matter population because the plants were picked randomly from the C_1 dry matter population without any selection. The greatest change occurred for heading date, where C_1 and C_2 dry matter populations had a significantly ($P < 0.0001$) 8-d earlier heading than the C_0 (Table 2.01) because dry matter yield in annual ryegrass is genetically correlated with heading date and fresh matter with a value of -0.986 and 0.996 (Fujimoto and Suzuki, 1975). For the remaining populations of C_2, (correlated traits), fresh weight population had shifted heading date slightly but significantly earlier than the heading date of the C_2 dry matter population. One probable reason behind early maturity might be the non-synchronized maturity in the isolation nurseries. Early heading tillers might have contributed more genes than late maturing because by the time late maturing tillers shed their pollen, most of the early tillers had already set their seed. Therefore recombination instead of selection might be the reason for the tendency toward earlier maturity.

Differences in distribution of heading date among three populations (C_0, C_1, C_2) are illustrated in Fig. 2.02. Data collection for heading date began on March 1, 2008 when there was 75 % emergence of the inflorescence from the boot in at least 5 spikes in at least one plant. After two years of selection, plants at the 50th percentile in the C_1 and C_2 populations both headed 13 days earlier than the base population. At the 90th percentile, C_1 and C_2 populations headed 7 days earlier in heading than the base population. At 99th percentile, C_1 headed earlier by 9 days and C_2 by 11 days as compared to the base population. As in fig.2.02, differences between C_1 and C_2 were observed only at the extreme percentiles (5th, 95th and 99th) which can be due to inclusion of late maturing plants.

Overall differences of cycles C_1 and C_2 from C_0 were of 7 and 8 days. C_1 and C_2 varied by 1 day only. Though the heading date became earlier with selection from C_0 to C_1 the change from C_1 to C_2 was very small, which is supported by Fig. 2.03.

By considering plants falling in the range of 5 to 95 percentile, differences observed among three populations were less while the inclusion of plants ranging from 1 to 99 percentile gave large variation among populations (Fig. 2.03). Thus most variation among populations occurred due to plants falling in extreme percentiles. It indicates that variation is explained by a small number of plants that were selected in the base population but excluded from C_1 because extremely late maturing selection did not contribute to the next cycle. Also, intra-population variation followed the same pattern in which the range for heading date within each population increased by including plants from 1 to 99 percentile. Base population had the highest intrapopulation range followed by C_1 and C_2, where C_1 and C_2 were almost the same. It means that the differences between C_0 and the other two cycles were due to the exclusion of extreme maturing plants in C_1 and C_2. The change in range from C_0 to C_1 was by 6-d and by 2-d from C_1 to

C_2 which suggests little change should be expected in further cycles. The heading range decreased and became almost the same for three cycles on the 5 to 95 percentile.

During the evaluation phase of each cycle, all plants were harvested uniformly at a height of 5 cm above ground because the main criterion was biomass production of individual plants without considering their plant type (erect or prostrate). Data was collected in seed yield nurseries during mid - March to get an accurate estimate of tiller angle because with time (age), zenith angle (angle with respect to vertical) increases resulting in plants with wide angles (Gibson et al., 1992). All C_2 populations became significantly ($P < 0.0001$) more erect than the base population as an indirect response to selection for dry matter yield. Prostrate plants with wide tiller angles hinder light interception by lower leaves, which results in less photosynthesis. Erect plants, on the other hand, have more leaf area exposed for light interception resulting in more photosynthate accumulation, which in turn increases dry matter production. During selection, plants with high dry matter were collected, which indirectly selected erect plants. Therefore, it can be the reason behind the erectness of plants. Various studies are being carried out in rice in which erectness, a QTL trait, is considered as a trait to increase the dry matter accumulation. But highly erect plants can also become more susceptible to diseases. In our nurseries, no population was perfectly erect and the maximum scale was 4.3. In annual ryegrass and meadow bromegrass, spread and height have shown nonsignificant relation with dry matter. On the other hand, height had a high genetic variance and is considered a highly heritable trait with high narrow and broad sense heritability (0.60-0.79) for selfed, polycross and open-pollinated progenies. Both narrow and broad sense heritabilities of height confirmed that the major part of variation was contributed by additive variance, whereas spread had low narrow sense heritability of 0.28-0.41.(de Araujo and Coulman, 2002; de Araujo and Coulman, 2004; Mittelmann et al., 2006). Moreover, plant height is negatively correlated with head emergence (Hazard et al., 2006). In indirect responses, percent dry matter and visual populations have shown slightly more erect growth but with more significant results than dry matter population (Table 2.02).

Maximum lignin content is reached when a plant attains its highest dry matter. Thus, with increase in dry matter, lignin content also increases, which in turn provides resistance to plant diseases (Taiz and Zeiger, 2006). In a study conducted in smooth bromegrass, low NDF and high NDF populations selected through mass selection affected dry matter yield but different disease severities of *Cochliobolus sativus*, the cause of leaf blight had no effect on dry matter production (Han et al., 2001).

No particular pattern was observed for disease occurrence. In seed increase nurseries, disease data was collected on individual plants and each plant was rated as diseased and non-diseased. Generally leaf spots were predominant but the approach was to take general observations about disease frequency without considering any particular disease. During analysis, average frequency per plot was calculated. All C_2 populations have shown an increase in incidence but with non-significant results (Fig. 2.04).

Seed yield was increased during two cycles of phenotypic recurrent selection (Table 2.03). Similarly, seed yield has shown a significant correlation with dry matter yield in meadow bromegrass (*Bromus riparious* Rehm.) (de Araujo and Coulman, 2002; de Araujo and Coulman, 2004). Tiller number is a seed yield component (Rhodes, 1973) and dry weight has a high genotypic and phenotypic correlation with tiller number

(Fujimoto and Suzuki, 1975), therefore selection for an increase in dry matter yield resulted in increased seed yield. The population selected for two cycles based on forage dry matter yield exceeded the base population by 88 kg ha^{-1} ($P < 0.001$). The population selected for dry matter yield in C_1, followed by random or fresh weight selection in C_2 exceeded the base population by 25% ($P \leq 0.002$). The remaining populations, although numerically larger did not differ significantly from the base population. The C_1 dry matter and the C2 visual populations had a significantly higher seed yield ($P \leq 0.025$) than the C_2 dry matter population.

Summary

All indirect responses, except disease reaction, had drastic modifications in cycle 1 but then became constant in cycle 2. Obviously there was genetic variation for these traits in the base population. A likely explanation for the drastic changes in heading date might simply be that cycle 1 selection included some very late-maturing plants that never contributed to the next generation because of non-synchronous flowering. Since isolation nurseries were bulk-harvested there is no way of knowing. But given the generally high heritability of reproductive maturity, four out of 43 selections not contributing might well have induced this change. Indirect selection criteria such as green matter or visual selected > 90% of the plants chosen on the basis of dry matter and thus present a cost-effective way to select for improved winter growth.

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Table 2.01: Effect of two cycles of phenotypic recurrent selection on heading date in annual ryegrass.

Population	Heading Date	Dunnett's *P*	Contrast *P* vs. C_2-Dry matter
C_0	29-Apr		
C_1-Dry matter	21-Apr	<0.0001	0.399
C_2-Dry matter	21-Apr	<0.0001	
C_2-Fresh weight	18-Apr	<0.0001	<0.0001
C_2-% dry matter	21-Apr	<0.0001	0.822
C_2-Visual	20-Apr	<0.0001	0.037
SED	0.8 d		

Table 2.02: Effect of two cycles of phenotypic recurrent selection for dry matter yield 750 GDD post transplanting on plant type in a spaced-plant nursery of annual ryegrass.

Population	Plant type	Dunnett's *P*	Contrast *P* vs. C_2-Dry matter
C_0	3.9		
C_1-Dry matter	4.2	<0.0001	0.704
C_2-Dry matter	4.2	<0.0001	
C_2-Fresh weight	4.3	<0.0001	0.009
C_2-% dry matter	4.3	<0.0001	0.021
C_2-Visual	4.3	<0.0001	0.081
SED	0.08		

Table 2.03: Effect of two cycles of phenotypic recurrent selection on spaced- plants seed yield in annual ryegrass.

Population	Seed yield	Dunnett's *P*	Contrast *P* vs. C_2-Dry matter
	-- kg ha^{-1}--		
C_0	452		
C_1-Dry matter	592	<0.0001	0.014
C_2-Dry matter	544	<0.0001	
C_2-Fresh weight	522	0.493	0.627
C_2-% dry matter	493	0.874	0.280
C_2-Visual	657	0.002	0.025
SED	38.7		

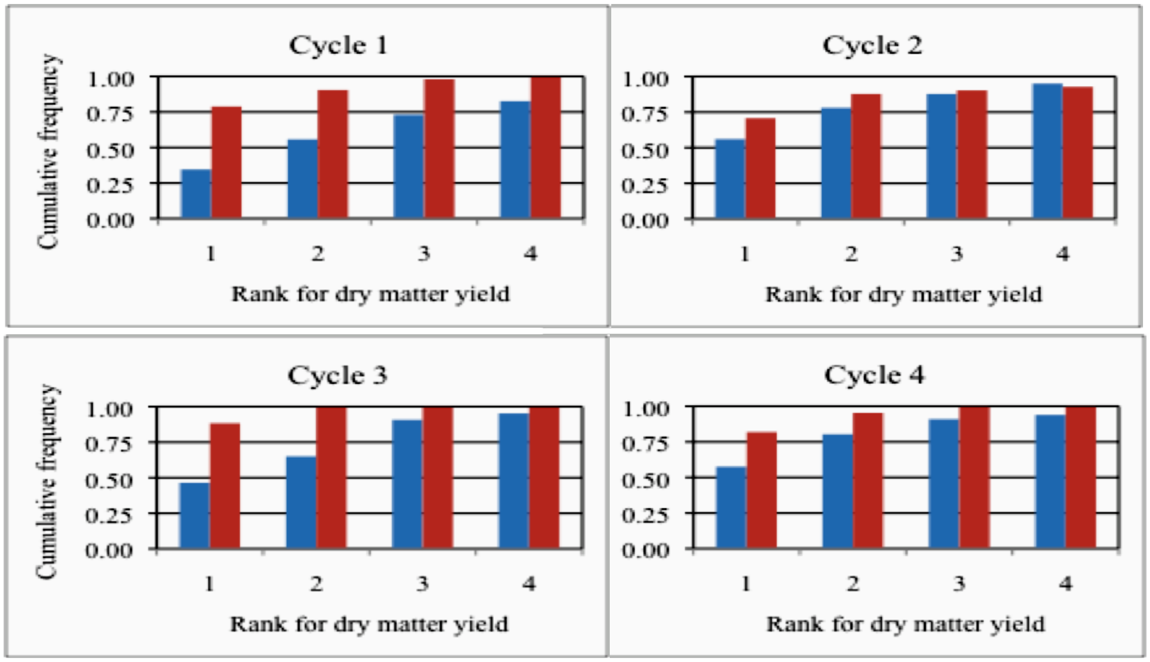

Figure 2.01: Cumulative frequency for selected plants (top ranked) based on visual (left columns) or green forage matter (right columns) in relation to the top four ranks for dry matter yield selection.

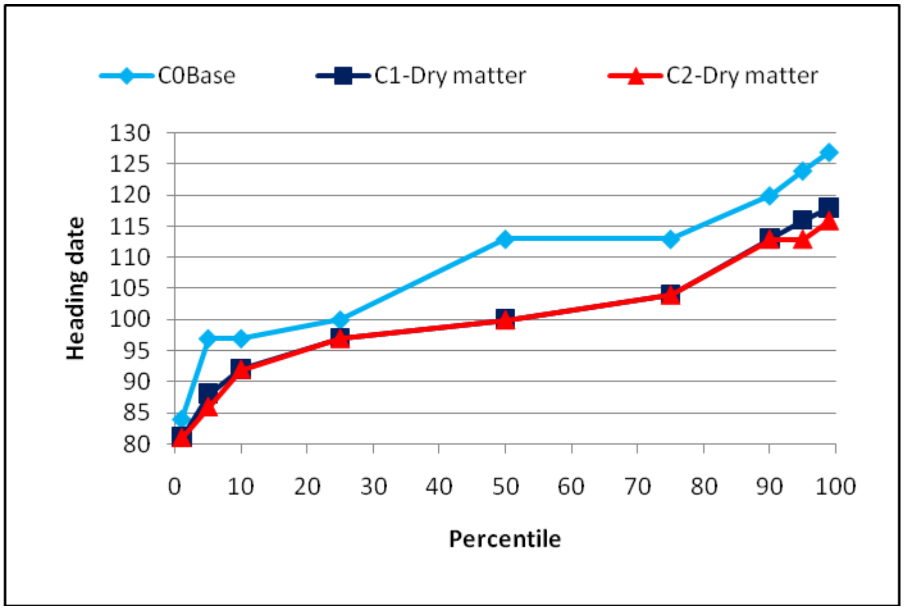

Figure 2.02: Percentile distribution of three populations (C0, C1-Dry matter, C2-Dry matter) for heading date after two cycles of phenotypic recurrent selection for dry matter yield in a spaced-plant nursery of annual ryegrass.

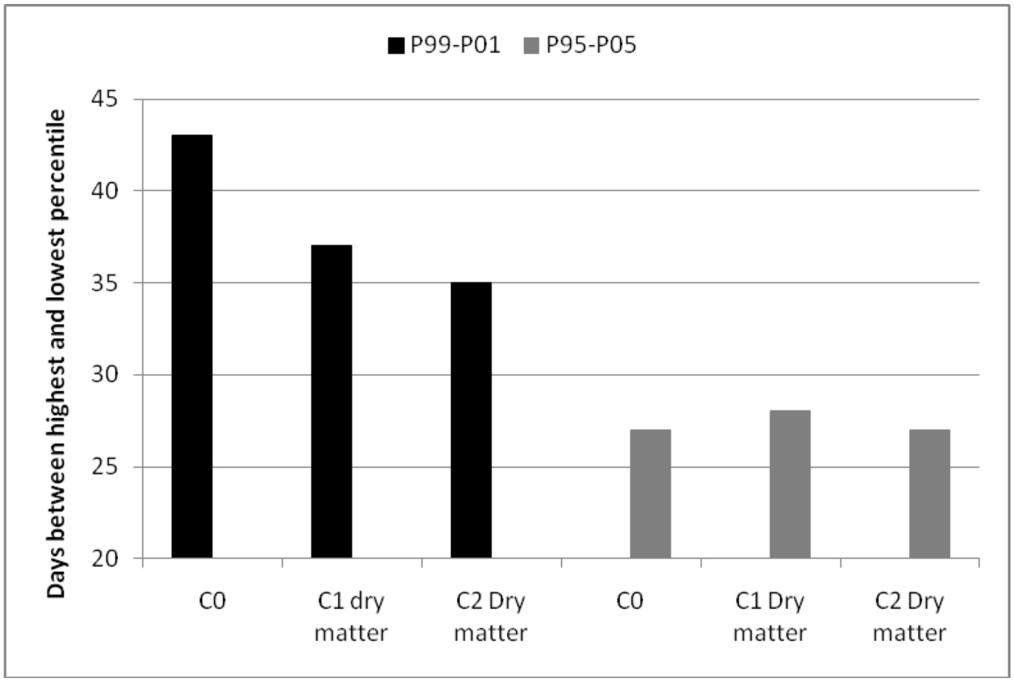

Figure 2.03: Intra-population variation within three cycles (C_0, C_1 Dry matter, C_2 Dry matter) at different percentile ranges. P99-P01 includes plants for heading date falling within range of 1to 99 percentile and P95-P05 includes plants within range of 5 to 95 percentile.

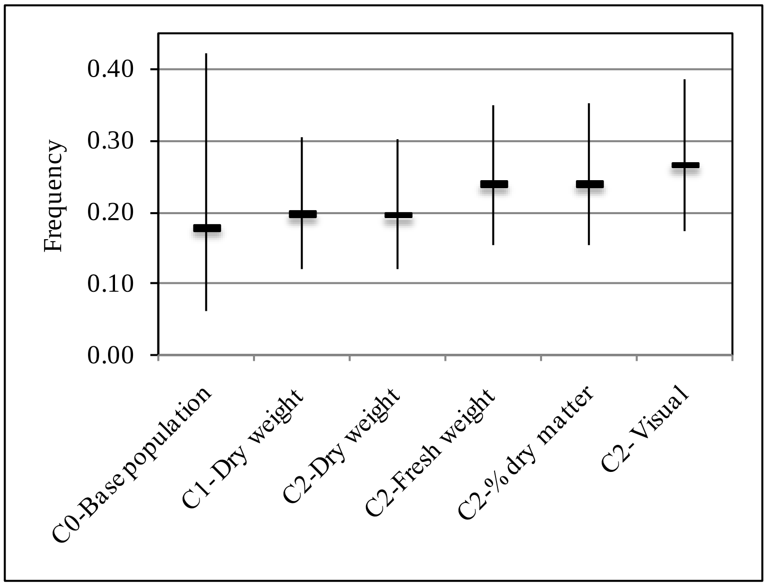

Figure 2.04: Effect of two cycles of phenotypic recurrent selection on disease frequency in a spaced-plant nursery of annual ryegrass.

III. EVALUATING PROGRESS FROM SELECTION FOR WINTER PRODUCTIVITY IN ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM* LAM.)

Abstract

Cool season annuals and perennials have lower forage production in autumn and winter compared to spring. Annual ryegrass (*Lolium multiflorum* Lam.) is a cool season forage crop with high dry matter production during the spring months. In order to make annual ryegrass available in forage deficit months, a breeding approach was followed to shift the seasonal yield distribution towards winter months. The base population (C_0) was generated by polycrossing six cultivars for two generations. C_0 was subjected to phenotypic recurrent selection for two cycles to increase the early winter productivity by selecting plants in late January or early February. The objective of this study was to evaluate the progress made with a 4.0 % selection intensity from selection for the first two cycles (C_1, C_2). The base population (C_0), and C_2 populations selected based on indirect responses (green matter, visual, percent dry matter), and three checks were included in the evaluation as well. The populations of two cycles (C_1, C_2) were compared with C_0 and check cultivars to assess progress. The experiment was conducted at five locations in Alabama under RCBD (r = 4). Three harvests were done on the basis of Growing Degree Days to measure the yield of each cut and total seasonal yield. GDD was calculated on the Celsius scale. For the first cut, selection resulted in a gain per cycle of 477 kg ha^{-1}, 396 kg ha^{-1} and 44 kg ha^{-1} at Tallassee, Headland and Belle Mina

respectively. The response was negative (-127 kg $ha^{-1}cycle^{-1}$) for Winfield and a negligible decrease (-8 kg $ha^{-1}cycle^{-1}$) at Fairhope. No significant deviation from a linear response was observed. During the second cut, there was a negative response to selection of -289 kg $ha^{-1}cycle^{-1}$, -241 kg $ha^{-1}cycle^{-1}$ and -52 kg $ha^{-1}cycle^{-1}$ at Belle Mina, Tallassee and Headland respectively, whereas there was an increase of 279 kg $ha^{-1}cycle^{-1}$ and 244 kg $ha^{-1}cycle^{-1}$ at Winfield and Fairhope respectively. Significant deviation from linearity was noticed at Belle Mina and Winfield. At the last harvest, the response became negative for all locations, with deviation from linearity at Belle Mina and Fairhope. More positive improvements were observed during the first cut because recurrent selection was carried out to improve early winter productivity; therefore it indicated the effectiveness of two cycles of selection. Moreover for the first cut, only Shiwasuaoba performed better than the C_2 population. Overall dry matter yield from C_0 to C_2, calculated as the sum of three cuts at each locations, has shown an increase of 239 kg $ha^{-1}cycle^{-1}$, 159 kg $ha^{-1}cycle^{-1}$ and 131 kg $ha^{-1}cycle^{-1}$ for Fairhope, Winfield, and Tallassee, respectively, while a negative response was observed for Belle Mina (-294 kg $ha^{-1}cycle^{-1}$) and Headland (-403 kg $ha^{-1}cycle^{-1}$). Indirect responses at all locations have shown insignificant differences from the C_2-dry matter population, which emphasizes the effectiveness of alternative methods of indirect selection through visual or green matter. Hence, two cycles of phenotypic recurrent selection resulted in improving early winter dry matter production.

Introduction

Annual ryegrass is a major cool season crop in the Southeastern USA, where it is grown on approximately one million hectares annually (Balasko et al., 1995). It has a high growth rate during the reproductive phase in spring but makes relatively little growth during winter. Availability of live forage for grazing in the southeastern USA is limited during the cooler months, and improved winter growth would be a great benefit to cattle producers. Therefore, the approach was to improve early winter dry matter production and to shift the seasonal yield distribution towards the cooler months. Phenotypic recurrent selection with grid selection was carried for two seasons (2006/07 and 2007/08) to increase the winter production during which plants were selected for the increased dry matter yield. This trait has low heritability, therefore to increase genetic gains per cycle, grid selection with high selection intensity (Burton, 1974) and selection before anthesis were performed.

Increase in dry matter yield is virtually always the key objective in forage breeding. Phenotypic recurrent selection procedures are most widely used for forage improvement because recombination is easy due to self incompatibility. Moreover, this method is easy to handle and apply. It improves low heritability traits by accumulating desirable alleles by keeping genetic variance constant. It involves evaluation and selection of desirable plants followed by recombination to create a base population for the next cycle.

Various high dry matter yielding forage cultivars have been developed through recurrent selection methods. Four cycles of recurrent selection with restrictions increased the forage yield by 16% and 19% in two populations (narrow gene pool and wide gene pool) of Pensacola bahiagrass (*Paspalum notatum* Flügge) (Burton, 1974), and 6 to 7% in rye (*Secale cereale* L.). Similarly, Marshall (Arnold et al., 1981) and Shiwasuaoba (Kindiger et al., 2004) cultivars of annual ryegrass were developed through recurrent selection. Four cycles of recurrent selection increased the level of resistance against brown leaf spot (caused by *Pyrenophora bromi* (Died) Drechs) in smooth bromegrass (*Bromus inermis* Leyss.) (Berg et al., 1986).

The phenotype of any plant is determined by its genotype (G), the environment (E) it is grown in and the G x E interaction, where environment may include different years or locations. The G x E component is unpredictable and poses a challenge for plant breeders. Multi-location yield trials allow genotype x location interaction to identify a cultivar suitable for a particular location and over years allows genotype x year interaction for different weather conditions because this factor is also unpredictable. Yield stability is the consistency of relative trait expression of a cultivar under different environments. The ideal cultivar would have acceptable performance in poor environments while responding positively to better environments. Yield stability is heritable and recurrent selection can result in increasing yield stability by selecting cultivars by their mean performance in different environments. Lack of yield stability has been the main reason behind inconsistent yield performance over a twelve year period in Louisiana, which suggests the desirability of multi-location evaluation of cultivars instead of single location testing in order to increase yield potential and stability in annual ryegrass (Redfearn et al., 2005).

A breeder conducts indirect selection when a direct response becomes difficult or expensive to measure during selection. While keeping constant selection intensity, indirect selection becomes more effective than direct selection only if $r_G h_2 > h_1$, where h_2 and h_1 are the narrow sense heritability for the indirect and direct response and r_G is the genetic correlation between the indirect and direct responses (Gallais, 1983). Dry matter is generally measured as a direct response for forage yield. Dry matter assessment involves harvesting, drying, weighing, and evaluation, which is laborious and time consuming. On the other hand, if a strong genetic correlation exists among dry matter yield, visual and fresh matter yield as is the case in perennial ryegrass, indirect selection through fresh matter yield results in evaluation of a greater number of genotypes as fresh matter selection is less labor intensive (Conaghan et al., 2008).

Thus objectives of our research were to (i) evaluate the progress made from the first two cycles of recurrent selection as compared to the base population and selected check cultivars, (ii) evaluate yield distribution of populations within growing season, and (iii) to compare dry matter yield of the population selected based on dry matter yield to populations selected based green matter, visual and dry matter percentage.

Materials and Methods

Base population

The base population was created from five high performing cultivars in the Alabama Annual Ryegrass Performance Trials. The cultivar Gulf was included as the sixth population based on its high performance in the Southeastern trials and because it has never been outyielded consistently by any newer cultivar. In autumn 2003, 50 plants from each cultivar were transplanted to a polycross nursery in the field under randomization and open pollination was allowed. In June 2004, seed was bulk harvested without any selection. In autumn 2004, a 300-plant nursery was established from bulk harvested seed of previous season (first synthetic generation). Again open pollination and no selection were practiced and seed was bulk harvested in June 2005. Two years of open pollination without any selection created the random mating base population (C_0).

Selection protocol

To start recurrent selection, the base (C_0) population was seeded into conetainers in August 2005. Conetainers were filled with a 1:1 mixture of peat based medium and sand. After two weeks of establishment, the seedlings were thinned to one seedling per conetainer. Plants were irrigated two times a day and before transplanting to field, the plants were fertilized once to promote growth. In early November, approximately 1200 plants were transplanted in blocks of 25 plants each. Weeds were controlled with glyphosate about two weeks before transplanting to avoid any weed competition. Plants were allowed to grow until the end of January or early February until they accumulated 750 GDD [C]. The basic purpose behind GDDs was to evaluate plants during early winter (which was our main objective) and select plants that were well established with high vigor and biomass production.

For selection cycle 1 (C_1), plants were selected only on the basis of dry matter yield (DM) but for cycle 2 (C_2), five types of selections were performed: visual estimation of yield, green matter (GM), DM, dry matter percentage (%DM) and random. The reason behind various selection schemes was to establish correlation between visual, GM, DM and % DM. After 750 GDD, two persons performed a visual estimation of forage yield and selected the best plant. Afterwards, all plants were harvested for green matter evaluation by collecting all tillers, either prostrate or erect. The gathered tillers were then cut at a uniform height of 5 cm using a wooden block as a height guide. Harvested samples were weighed to determine their fresh matter and then dried at 60 C for 3 days. Dried samples were weighed again to measure dry matter production of each plant. Based on GM and DM, % DM was calculated as ratio of DM to GM. After evaluation, one plant per block (4% selection intensity) was selected for each criterion where random selection was done by picking one plant randomly from each block without any selection. Approximately two weeks after harvest, selected plants were dug up and transplanted back in a greenhouse for two weeks. Some plants were selected for more than one selection criteria. So accordingly, plants were split and established in different pots for root establishment.

After root establishment, these five populations were transplanted to isolated nurseries for recombination and to generate seed for the next cycle. Due to non-synchronized maturity, plants were harvested at short intervals (at a gap of 2-3 days) to harvest each whole isolated nursery.

Replicated spaced plant seed increase

The base population along with five populations from C_2 of the phenotypic recurrent selection program for winter productivity was planted in a greenhouse during late August, 2007. The C_2 populations included dry matter, fresh weight, dry matter percentage, visual, and random populations. C_0, C_1 and C_2 were represented by the base population, the random population of C_2 and dry matter of C_2, respectively. Seeds were sown into 800 conetainers (supplier +location) per population containing 1:1 sand and Pro Mix. Plants were thinned to one plant per conetainer two weeks after seeding. In early November, the six populations were transplanted into replicated seed increase nurseries (RCBD, r = 4). Each plot consisted of 200 plants on 90-cm centers. Plots were surrounded with 10 m borders of rye (*Secale cereale* L.) to avoid cross-pollination among populations. Plants were harvested during late May 2008, when the rachis below the inflorescence turned brown. All 24 (6 populations × 4 replications) plots were harvested separately with bulking of seed within each plot. Bulk harvested seed per plot was used to calculate seed yield.

Multi-location yield trials

In 2008, the 24 populations harvested from replicated seed increase nurseries along with three checks were planted at five locations in Alabama (Fig. 3.01) at rate of 22.4 kg ha^{-1}. Gulf and Marshall are long term checks used in cultivar performance trials throughout the southern USA that have never been consistently outyielded by any newer cultivar. Gulf, released in 1958 by Texas USDA-AES has higher resistance for crown rust and yield ability than common ryegrass. It has high forage yield in the southern parts of the USA (from east Texas to Florida). It is an early maturing cultivar well adapted to coastal areas of Texas and Louisiana. It has high value for early fall and winter growth and seed yield (Weihing, 1963). Marshall was released by Mississippi State University in 1980 and has high cold tolerance and seedling vigor. It is a late maturing cultivar, maturing about two weeks later than Gulf, hence providing forage for a longer period in spring than other diploid species (Arnold et al., 1981). Shiwasuaoba is an early maturing cultivar with early fall and spring production (Kobashi et al., 1998), with seed head emergence two to three weeks earlier than Marshall and perennial ryegrass. Early head emergence makes it a useful cool season cultivar for early spring production with high nutrition and palatability. It was evaluated for adaptation and performance in the USA, where its total yearly biomass was 60% of Marshall (Kindiger et al., 2004). Therefore all these checks provide a basis for comparison of early, mid and late yield distribution.

The plot dimension for the trial conducted as an RCBD (r = 4) was 3 x 1.25 m, with a tractor-tire width gap between adjacent plots. To minimize the within-block variation we chose the most compact arrangement of plots with three plots in the planting direction, separated by a 1.8-m alley, and nine plots across. Nitrogen was applied at a rate of 56 kg ha^{-1} at the sowing and after each harvest. This assured that N was not limiting plant growth thus potentially maximizing differences among entries. The first harvest was done at 1000 GDD (Growing Degree Days) including 250 GDD for initial emergence and remaining 750 for dry matter accumulation. Later cuts were done at intervals of 750 GDD. Seeding and harvesting dates of three cuts are given in table 3.01.

Data was collected for plot green weight, sample green weight and sample dry weight. Plot yield, calculated as Plot GW*Sample DM/Sample GW, was converted to dry matter per hectare.

Statistical Analysis

We used nearest neighbor analysis (NNA) to adjust observed plot values for individual harvests as well as the cumulative seasonal yield. A randomized complete block design (RCB) can result in variation within blocks despite blocking to provide homogenous environment. Variation can occur due to soil fertility, disease frequency, weed infestation etc. Even with the most compact arrangement of plots within blocks, some variation still exists within blocks, which decreases precision of method (Smith and Casler, 2004).

In NNA analysis the yield potential of a given plot is calculated from the residuals of neighboring plots (Brownie et al., 1993). Pre-adjustment analysis of NNA is applied on individual harvests. NNA reduces the value of the Least Significant Difference (LSD) and Least Significant Range (LSR). Decreased LSD and LSR values improve the precision and detect more differences among cultivars (Smith and Casler, 2004). We therefore used a row covariate (average of residuals of the two neighboring plots within a row), column covariate (average of residuals of the two neighboring plots within a column), or a total covariate (average of residuals of all four neighboring plots), which accounts for residuals along the boundaries of plots, to adjust observed plot yields for spatial variation.

The three covariates were considered fixed factors along with population, whereas block and seed increase block within population were random factors. For each location x harvest combination we fitted four models (population, row covariate + population, column covariate + population, and total covariate + population). The fit statistics from these models were collected and subjected to a ranking procedure (PROC RANK) to identify the model with the lowest AICC value. This fit statistic is the negative log likelihood penalized for the number of terms and akin in intent to an adjusted coefficient of determination in least squares regression. The model with the minimum AICC value was selected as the adjustment in the final analysis of variance.

First and second order polynomial contrasts were used to evaluate the effect of selection cycle on dry matter yield. Linear contrasts were used to find the relationship between indirect responses and C_2 dry matter response. Similarly, two sets of contrasts were constructed to (1) estimate the differences between the base population and the three check cultivars and (2) estimate the differences between the C_2 dry matter population and the three check cultivars. As indicated earlier, these check cultivars were selected because of their differing seasonal dry matter distribution.

Results and Discussion

Dry matter yield is a complex trait with low heritability (Fujimoto and Suzuki, 1975). Evaluation of recurrent selection was carried out at five locations. These locations were distributed from north to south Alabama to provide diverse environments for evaluations.

Harvest 1

A significant ($P \leq 0.007$) linear increase in first-harvest yield due to selection for improved winter productivity was obtained for Tallassee, the selection location in Central Alabama, and Headland, located in southeastern Alabama (Table 3.02). First-cut yields increased by 954 kg DM ha^{-1} over two cycles at Tallassee and 792 kg DM ha^{-1} at Headland. The response at the northernmost location (Belle Mina) and the southernmost (Fairhope) were non-significant ($P \geq 0.417$). Dry matter yield at Winfield declined by 127 kg ha^{-1}cycle^{-1} ($P = 0.067$). An explanation for the response at Winfield may be that it took five months to accumulate 1000 GDD, whereas at Tallassee and Fairhope it took 3 and 2.5 months respectively. The zero response at Fairhope may be explained by the fact that the 5-day rolling average daily low temperature never dropped below 4 C and remained between 10 to15 C most of the time. Growth of annual ryegrass can come to a halt when temperature reaches 6 C and becomes maximal in production at a temperature of 18 C (Weihing, 1963). No significant ($P \geq 0.416$) deviations from linearity were observed.

The objective for including indirect selection in cycle 2 was to evaluate alternate methods of selection for high dry matter yield. The evidence from the literature indicated a strong correlation of dry matter yield with visual estimation of yield or green matter. Our results confirm those findings because only a single contrast was significant; the population selected visually in C_2 yielded 387 kg DM ha^{-1} more ($P = 0.002$) than C_2 dry matter at Belle Mina (Table 3.03).

Shiwasuaoba gave the highest first-cut yield at all locations. Shiwasuaoba had significantly higher dry matter yield than the base population at three locations (Tallassee, Headland, Fairhope), which have mild to high temperatures. This was the expected behavior of Shiwasuaoba as it was developed as an early maturing cultivar (Table 3.04). In northern locations, Shiwasuaoba gave high yield but with non-significant differences at Belle Mina ($P = 0.075$) and Winfield ($P = 0.260$), which might be due to cool temperatures; Shiwasuaoba is recommended at places with temperature above 16 C in October (Kindiger et al., 2004), which indicates its adaptability at high temperature locations. For the remaining two checks, differences were insignificant except for Marshall at Headland ($P = 0.022$). Gulf and Marshall varied in their response across locations.

Rankings for the three checks and C_2 dry matter followed a geographic pattern. For the northern two locations it was Shiwasuaoba > Marshall > C_2 DM > Gulf; for the two central locations (Tallassee, Headland) Shiwasuaoba > C_2 DM > Gulf > Marshall; and for the southernmost location Shiwasuaoba > Marshall > C_2 DM > Gulf. In Auburn University ryegrass performance trials Shiwasuaoba yielded near 47-51% of its production in autumn at Tallassee and Wiregrass and 37% at Fairhope (Glass and van

Santen, 2008). The C_2 DM population significantly outyielded Marshall and Gulf at the central Alabama locations (Table 3.05).

Harvest 2

Harvest 2 evaluates the indirect effects of selection for improved winter productivity on regrowth. Results were mixed ranging from a decline of 289 kg DM ha^{-1} $cycle^{-1}$ (P = 0.001) at Belle Mina to a 279 kg DM ha^{-1} $cycle^{-1}$ (P = 0.062) improvement at Winfield (Table 3.06); the latter showed a stronger increase in C_1 compared to C_2, leading to a significant deviation from linearity (P = 0.003).

All three indirect selection methods have shown non-significant differences from C_2-dry matter with a few exceptions such as visual (P = 0.001) and percent dry matter (P = <0.001) at Belle Mina where the former gave lowest yield by -565 kg $ha^{-1}cycle^{-1}$and the latter gave the highest yield by 888 kg $ha^{-1}cycle^{-1}$ (Table 3.07). At Fairhope, percent dry matter (P = 0.003) and green matter (P = 0.031) gave a significantly high dry matter yield of 617 kg ha^{-1} and 418 kg ha^{-1} than C_2 dry matter. Therefore these responses again supported indirect selection as a substitute for selection on dry matter basis.

Shiwasuaoba yielded the least dry matter (P = 0.001 to 0.004) at all locations, except at Fairhope where Shiwasuaoba gave the highest yield (4589 kg ha^{-1}) but with non-significant differences (P = 0.140) from C_0 (Table 3.08). During the first harvest Shiwasuaoba gave more dry matter yield than C_0 at difference of 2174 kg ha^{-1}, and 2766 kg ha^{-1} at Tallassee, Headland respectively (Table 3.04). In the second harvest, C_0 yielded more than Shiwasuaoba for dry matter yield with difference of 1499 kg ha^{-1}, 3122 kg ha^{-1} at Tallassee and Headland respectively. Thus, the decline in Shiwasuaoba was greater at Headland.

On comparing C_2-dry matter population with the three check cultivars (Table 3.09), Shiwasuaoba gave the lowest yield at all locations except Fairhope. Gulf performed better at Belle Mina whereas Marshall gave more dry matter yield than C_2-dry matter at Belle Mina and Headland.

Harvest 3

Third harvest dry matter yield decreased from C_0 to C_2 at all the locations (Table 3.10) with significant decreases at Belle Mina (P = 0.048), Fairhope (P = 0.057) and Headland (P = 0.002). At Belle Mina and Fairhope, three cycles have shown non-linear response to selection with P = 0.04 and 0.001. At all the locations, percent decrease from C_0 to C_2 was 4%, 5.2%, 7.6%, 14% and 23.3% with a decline of -75 kg $ha^{-1}cycle^{-1}$, -79 kg $ha^{-1}cycle^{-1}$, -404 kg $ha^{-1}cycle^{-1}$, -200 kg $ha^{-1}cycle^{-1}$and -404 kg $ha^{-1}cycle^{-1}$at Fairhope, Belle Mina, Tallassee, Winfield, and Headland respectively. This decrease might have occurred because for the first two cycles, selection was done for high dry matter yield in early winter months.

The C_2-dry matter and all indirect responses performed similarly (Table 3.11) except at Fairhope where percent dry matter gave the highest dry matter yield (P = <0.001). These non-significant differences again strongly recommend the alternative method either on the basis of visual or green matter.

As expected, the dry matter yield of Shiwasuaoba significantly declined at all the locations except at Tallassee where decrease was non-significant. Gulf outyielded C_0 at Fairhope (P = <0.001) and Headland (P = 0.014). Marshall performed better than C_0 at Belle Mina (P = 0.007) and Fairhope (P = <0.001) (Table 3.12).

For C_2-dry matter yield (Table 3.13), again Shiwasuaoba had a lower yield and contrasts followed the same pattern as that of C_0. Marshall yielded more dry matter than C_2-dry matter at Belle Mina (P = 0.023) and Fairhope (P = <0.001). Gulf also performed better than the C_2-dry matter at Fairhope (P = <0.001).

Total seasonal yield

Total seasonal yield was calculated across the five locations by taking the sum of all three harvests (1, 2, 3) and the same statistical analysis was used.

Total dry matter yield was high at all locations except Belle Mina, where yield was high for all three cycles (Table 3.14). At Belle Mina, linear contrast showed a significant (P = 0.007) decline of -294 kg ha^{-1} $cycle^{-1}$ in response to selection from C_0 to C_2. The deviation contrast (quadratic term) was also significant as well as positive, indicating that most of the decline happened during the first cycle. Yield increased from C_0 to C_2 by 4.6% with a gain of 239 kg ha^{-1} $cycle^{-1}$ at Fairhope. Decline was <1% at Winfield and Tallassee. Total seasonal yield declined at Headland by 4%. But at Winfield, Tallassee and Fairhope, both linear and quadratic contrasts showed non-significant response for selection (Table 3.14).

Indirect responses showed insignificant differences from C_2-dry matter population at all the locations except at Belle Mina and Fairhope where percent dry matter gave significant differences from C_2-dry matter (Table 3.15). Therefore considering all three cuts and total yield, we can easily make an inference that visual and green matter can be effectively used as indirect selection criteria for selecting the plants for high dry matter yield. It can result in the selection of a greater number of plants as both indirect criteria involve less labor and time (Mittelmann et al., 2006).

A similar response was observed for the base (Table 3.16) and C_2-dry matter populations (Table 3.17) when compared with the three checks. Gulf outyielded C_0 and C_2-dry matter only at Belle Mina and Shiwasuaoba yielded less at Headland.

On comparing seasonal yield distribution of C_2 and check cultivars (Table 3.18), C_2 population has shown a relatively high percentage of dry matter yield in the fall season as compared to Gulf and Marshall, but yield was lower than Shiwasuaoba.

Summary

For the first cut, dry matter yield increased at three locations but not at Winfield and Fairhope due to low and high temperature conditions. Shiwasuaoba was the only check cultivar that performed better than the C_2-dry matter population. For the second cut, response to selection became positive only at Winfield and Fairhope. Yield of Shiwasuaoba declined and Gulf and Marshall gave significantly high yield at two locations. During the third harvest, yield decreased at all locations and Gulf and Marshall outperformed C_2 dry matter population at two locations only. For all three harvests, indirect responses have shown insignificant differences from C_2-dry matter.

For total yield, Headland gave the highest yield followed by Tallassee and Fairhope. Decline in yield was significant only at Belle Mina where quadratic deviation was significant. Shiwasuaoba gave the lowest total seasonal yield as compared to the C_0 and C_2-dry matter populations. Only Marshall performed better than C_0 and C_2 populations, and this occurred at only one location i.e. Belle Mina.

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Table 3.01: Seeding and harvesting dates at five locations during evaluation process of two cycles along with base and check cultivars.

	Belle Mina	Winfield	Tallassee	Headland	Fairhope
Seeding	9-Sep	24-Sep	11-Oct	30-Oct	29-Oct
Harvest 1	9-Dec	6-Mar	13-Jan	5-Feb	12-Jan
Harvest 2	2-Apr	24-Apr	11-Mar	9-Apr	20-Mar
Harvest 3	10-May	24-June	22-Apr	19-May	22-Apr

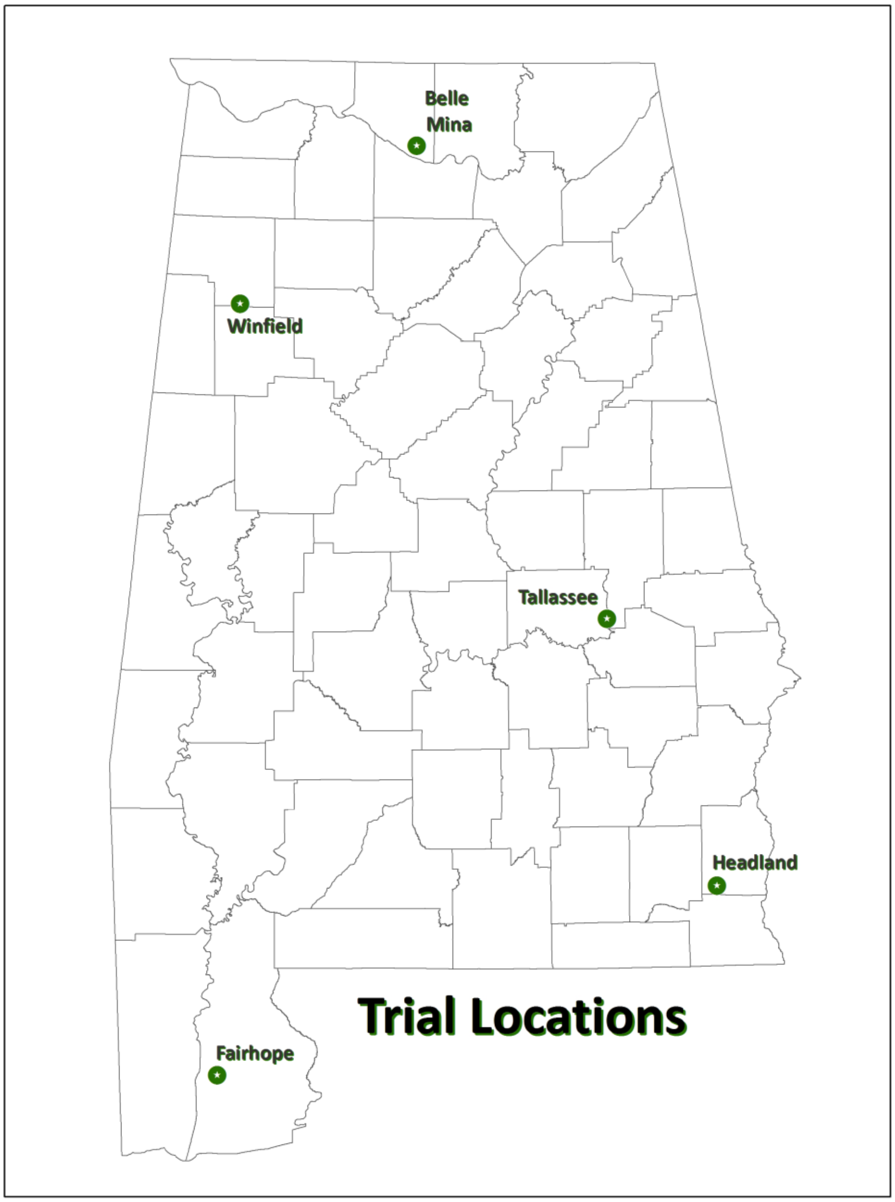

Figure 3.01: Five locations in Alabama selected to evaluate the progress made from first two cycles of phenotypic recurrent selection along with base population and check cultivars.

Table 3.02: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Means and orthogonal polynomial contrasts for harvest 1 at five evaluation locations.

Cycle	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	----------	----------	kg ha^{-1}	----------	----------
C_0	1436	1226	2337	3102	2957
C_1	1458	1153	2992	3473	2950
C_2	1525	972	3290	3894	2940
SE	126	104	221	186	77
<u>Selection cycle contrasts</u>					
Linear	44	-127	477	396	-8
SE	53	65	124	131	41
P-value	0.417	0.067	0.001	0.007	0.841
Deviation	22	-54	-179	25	-2
SE	91	113	215	227	70
P-value	0.810	0.636	0.416	0.913	0.980

Table 3.03: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Evaluation of indirect selection criteria for harvest 1 at five evaluation locations.

Cycle 2 population	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	---------------------------------- kg ha^{-1}	----------------------------------			
Dry Matter	1525	972	3290	3894	2940
Green Weight	1597	1179	2828	3492	2977
Visual	1912	1075	3035	3884	3033
Percent DM	1490	1049	3123	3531	3102
SE	126	104	221	186	77
Contrast for indirect selection response relative to dry matter					
Green Weight	0.503	0.129	0.079	0.144	0.655
Visual	0.002	0.438	0.320	0.970	0.276
Percent DM	0.748	0.561	0.509	0.183	0.063

Table 3.04: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Performance of the base population and three check cultivars for harvest 1 at five evaluation locations.

Population/ cultivar	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	----------------------------------	---------	kg ha^{-1}	---------	------------------------------
Base	1436	1226	2337	3102	2957
Gulf	1313	791	2366	3100	3047
Marshall	1664	988	2234	2435	3104
Shiwasuaoba	1774	1393	4511	5868	4063
SE	126	104	221	186	77
Contrast vs. Base					
Gulf	0.395	0.099	0.127	0.380	0.462
Marshall	0.233	0.433	0.070	0.022	0.249
Shiwasuaoba	0.075	0.260	0.001	< 0.001	< 0.001

Table 3.05: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Performance of the C_2 dry matter population and three check cultivars for harvest 1 at five evaluation locations.

Population/ cultivar	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	------------------	------------------	kg ha^{-1}	------------------	------------------
C_2 dry matter	1525	972	3290	3894	2940
Gulf	1313	791	2366	3100	3047
Marshall	1664	988	2234	2435	3104
Shiwasuaoba	1774	1393	4511	5868	4063
SE	181	192	375	372	126
Contrast vs.C_2 dry matter					
Gulf	0.221	0.398	0.030	0.071	0.417
Marshall	0.415	0.936	0.015	0.002	0.221
Shiwasuaoba	0.153	0.056	0.006	< 0.001	< 0.001

Table 3.06: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Means and orthogonal polynomial contrasts for harvest 2 at five evaluation locations.

Cycle	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	---------------------------------- kg ha^{-1} ------------------------------------				
0	4158	7893	3369	11596	3633
1	3553	9018	3251	11636	4151
2	3581	8451	2887	11491	4122
SE	167	191	181	336	136
Selection cycle contrast					
Linear	-289	279	-241	-52	244
SE	73	140	127	216	90
P-value	0.001	0.062	0.074	0.812	0.014
Deviation	316	-846	-123	-92	-274
SE	126	244	221	375	156
P-value	0.022	0.003	0.585	0.809	0.097

Table 3.07: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Evaluation of indirect selection criteria for harvest 2 at five evaluation locations.

Cycle 2 population	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	-------------------------------	------------	kg ha^{-1}	------------	-------------------------------
Dry Matter	3581	8451	2887	11491	4122
Green Weight	3668	8164	3011	12143	4540
Visual	3016	7886	2868	11700	3960
Percent DM	4469	8392	3273	12007	4739
SE	167	191	181	336	137
Contrast for indirect selection response relative to dry matter					
Green Weight	0.558	0.321	0.631	0.150	0.031
Visual	0.001	0.059	0.941	0.635	0.380
Percent DM	< 0.001	0.836	0.147	0.250	0.003

Table 3.08: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Performance of the base population and three check cultivars for harvest 2 at five evaluation locations.

Population/ cultivar	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	------------------------------- kg ha^{-1} -----------------------------				
Base	4158	7893	3369	11596	3633
Gulf	4120	7783	3226	11291	4089
Marshall	4578	7989	2976	9598	3633
Shiwasuaoba	1345	7541	1870	8474	4589
SE	167	191	181	336	136
Contrast vs. Base					
Gulf	0.024	0.014	0.951	0.620	0.830
Marshall	< 0.001	0.033	0.504	0.009	0.084
Shiwasuaoba	< 0.001	0.004	0.003	< 0.001	0.140

Table 3.09: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Performance of the C_2 dry matter population and three check cultivars for harvest 2 at five evaluation locations.

Population/ cultivar	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	-------------------------------	-------	kg ha^{-1}	-------	-------------------------------
C2 dry matter	3581	8451	2887	11491	4122
Gulf	4120	7783	3226	11291	4089
Marshall	4578	7989	2976	9598	3633
Shiwasuaoba	1345	7541	1870	8474	4589
SE	245	399	360	627	260
Contrast vs.C2 dry matter					
Gulf	0.031	0.153	0.411	0.773	0.911
Marshall	< 0.001	0.312	0.827	0.013	0.102
Shiwasuaoba	< 0.001	0.055	0.021	< 0.001	0.117

Table 3.10: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Means and orthogonal polynomial contrasts for harvest 3 at five evaluation locations.

Cycle	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	----------	----------	kg ha^{-1}	----------	----------
0	3038	2836	10576	3461	3785
1	2816	2955	9712	3036	3440
2	2880	2436	9768	2653	3635
SE	51	138	359	154	79
Selection cycle contrast					
Linear	-79	-200	-404	-404	-75
SE	37	103	243	114	37
P-value	0.048	0.068	0.113	0.002	0.057
Deviation	143	-319	460	21	270
SE	64	179	419	197	64
P-value	0.040	0.092	0.287	0.916	0.001

Table 3.11: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Evaluation of indirect selection criteria for harvest 3 at five evaluation locations.

Cycle 2 population	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	----------------------------------	---------------- kg ha^{-1}	----------------	--------------------	------------------------------
Dry Matter	2880	2436	9768	2653	3635
Green Weight	2853	2695	9312	2130	3698
Visual	2854	2566	9605	2561	3564
Percent DM	2973	2534	10608	2390	4150
SE	51	137	358	154	80
Contrast for indirect selection response relative to dry matter					
Green Weight	0.719	0.225	0.358	0.033	0.404
Visual	0.733	0.538	0.740	0.691	0.340
Percent DM	0.227	0.642	0.100	0.261	< 0.001

Table 3.12: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Evaluation of indirect selection criteria for harvest 3 at five evaluation locations.

Population/ cultivar	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	----------------------------	----------------	kg ha^{-1}	----------------	----------------------------
Base	3038	2836	10576	3461	3785
Gulf	2865	2654	10026	2057	4491
Marshall	3172	3351	10737	3031	4645
Shiwasuaoba	1639	2150	8437	1156	2570
SE	51	138	359	154	79
Contrast vs. Base					
Gulf	0.685	0.372	0.687	0.014	< 0.001
Marshall	0.007	0.241	0.197	0.990	< 0.001
Shiwasuaoba	< 0.001	0.024	0.114	< 0.001	< 0.001

Table 3.13: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Performance of the C_2 dry matter population and three check cultivars for harvest 3 at five evaluation locations.

Population/ cultivar	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	----------------------------------	---------- kg ha^{-1}	----------	----------	----------------------------------
C2 dry matter	2880	2436	9768	2653	3635
Gulf	2865	2654	10026	2057	4491
Marshall	3172	3351	10737	3031	4645
Shiwasuaoba	1639	2150	8437	1156	2570
SE	105	290	695	318	121
Contrast vs.C2 dry matter					
Gulf	0.898	0.055	0.741	0.114	< 0.001
Marshall	0.023	0.055	0.222	0.307	< 0.001
Shiwasuaoba	< 0.001	0.055	0.100	0.001	< 0.001

Table 3.14: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Means and orthogonal polynomial contrasts for total seasonal yield at five evaluation locations.

Cycle	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	----------------------------------	-------	kg ha^{-1}	-------	----------------------------------
0	8607	11997	16088	18330	10300
1	7929	12786	15997	17756	10441
2	8019	11875	15991	17523	10778
SE	135	356	641	511	226
Selection cycle contrast					
Linear	-294	-61	-49	-403	239
SE	96	244	401	375	164
P-value	0.007	0.806	0.905	0.297	0.161
Deviation	384	-850	43	171	97
SE	167	422	321	650	284
P-value	0.034	0.059	0.951	0.796	0.736

Table 3.15: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Evaluation of indirect selection criteria for total seasonal yield at five evaluation locations.

Cycle 2 population	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	---------------------------------- kg ha^{-1} ---------------------------------				
Dry Matter	8019	11875	15991	17523	10778
Green Weight	8020	11923	14990	17781	11364
Visual	7693	11599	15705	18525	10652
Percent DM	9091	12059	17045	18327	11963
SE	135	356	641	511	227
Contrast for indirect selection response relative to dry matter					
Green Weight	0.999	0.923	0.228	0.735	0.091
Visual	0.108	0.578	0.727	0.198	0.705
Percent DM	< 0.001	0.711	0.206	0.298	0.002

Table 3.16: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Performance of the base population and three check cultivars for seasonal yield at five evaluation locations.

Population/ cultivar	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	----------	----------	kg ha^{-1}	----------	----------
Base	8607	11997	16088	18330	10300
Gulf	8429	11887	16328	17155	11263
Marshall	9344	12395	15997	15408	11276
Shiwasuaoba	4374	11322	14179	14200	11210
SE	135	356	641	511	226
<u>Contrast vs. Base</u>					
Gulf	0.117	0.258	0.798	0.619	0.130
Marshall	< 0.001	0.617	1.000	0.064	0.124
Shiwasuaoba	< 0.001	0.073	0.169	0.008	0.156

Table 3.17: Evaluation of progress from two cycles of phenotypic recurrent selection at Tallassee, AL for dry matter production 750 GGD post transplanting: Performance of the C_2 dry matter population and three check cultivars for seasonal yield at five evaluation locations.

Population/ cultivar	Belle Mina	Winfield	Tallassee	Headland	Fairhope
	----------	----------	kg ha^{-1}	----------	----------
C2 dry matter	8019	11875	15991	17523	10778
Gulf	8429	11887	16328	17155	11263
Marshall	9344	12395	15997	15408	11276
Shiwasuaoba	4374	11322	14179	14200	11210
SE	273	695	1179	1053	460
Contrast vs. C2 dry matter					
Gulf	0.195	0.988	0.795	0.760	0.361
Marshall	< 0.001	0.509	0.996	0.092	0.349
Shiwasuaoba	< 0.001	0.482	0.170	0.012	0.415

Table 3.18: Seasonal yield distribution of C_2 and three check cultivars during evaluation of progress from two cycles of phenotypic recurrent selection across five locations.

Locations	Populations	Fall	Spring	Late sp.
		---------- % of total seasonal yield -------		
Belle Mina	C2	19	45	36
Belle Mina	Gulf	16	49	34
Belle Mina	Marshall	18	49	34
Belle Mina	Shiwasuaoba	41	31	37
Winfield	C2	8	71	21
Winfield	Gulf	7	65	22
Winfield	Marshall	8	64	27
Winfield	Shiwasuaoba	12	67	19
Tallassee	C2	21	18	61
Tallassee	Gulf	14	20	61
Tallassee	Marshall	14	19	67
Tallassee	Shiwasuaoba	32	13	60
Headland	C2	22	66	15
Headland	Gulf	18	66	12
Headland	Marshall	16	62	20
Headland	Shiwasuaoba	41	60	8
Fairhope	C2	27	38	34
Fairhope	Gulf	27	36	40
Fairhope	Marshall	28	32	41
Fairhope	Shiwasuaoba	36	41	23