Measuring Phenotypic and Genetic Variances and Narrow Sense Heritability in Three Populations of Annual Ryegrass (*Lolium multiflorum* Lam.)

by

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Abstract

Annual ryegrass, also known as Italian ryegrass is a cool season bunch grass supposed native to Italy and belongs to family Poaceae. Annual ryegrass is a short duration grass chiefly used for pasture and silage in dairy and beef cattle production. Due to low availability of live forage during early winters, beef and cattle producers rely on stored forage to meet nutritional requirements of animals, so increasing early winter productivity would be a great benefit for the beef industry. In order to increase early winter productivity, a recurrent selection project for improved winter dry matter productivity was initiated in which plants were evaluated and selected on the basis of dry matter yield 750 GDD post transplanting. As a common practice in forage breeding programs for cross-pollinated, non-domesticated species, we simply assumed that there is genetic variation for the traits of interest and no assessment of heritable variation was made before the start of the recurrent selection program. Yet it is of interest to investigate phenotypic and genetic variances and covariances in base and selected populations. To measure genetic variation and heritability for dry matter yield three half-sib populations, representing three cycles of recurrent selection were selected. In year 2008/09 the trial simulated a sward and was conducted only at the Plant Breeding Unit (PBU) of the Alabama Agricultural Experiment Station’s E.V. Smith Research Center in Tallassee, Alabama while in year 2009/10 the spaced planted trial was conducted at two locations in Alabama: PBU and the Alabama Agricultural Experiment Station’s Wiregrass Research and Extension Center at Headland (WGS). The experimental design for each trial was a randomized complete block (r = 2) with a split-split plot
randomization restriction (SSP). Three harvest schemes were employed. Two schemes were based on accumulated thermal degree-days (GDD) and the third based on heading date. Maximum likelihood methods were used to calculate variances and covariances, which were then used to estimate heritability for dry matter yield. First and second cut data for the first and second harvest scheme enabled us to evaluate genetic variation for productivity under autumn/winter conditions. The third harvest scheme with harvest at heading enabled us to evaluate the effect of maturity differences on genetic variation for yield. In year 2009/10 only two cuttings were done. First harvest was done at 500 GDD and second harvest was done at maturity. Considerable genetic variation among the three populations was observed. Generally the heritability values have been observed higher for year 2008/09 than 2009/10, for corresponding harvests. In both years the heritability values decreased in most of the cases with the each subsequent cycle but differences among the values were not significant, since the standard error values overlapped. Similarly heritability within harvest scheme also decreased with subsequent cuts within each population, but differences among values were also not significant. The trend for the values within harvest scheme can be explained due to increase in temperature in each subsequent cut, since the populations were produced from the selection for high winter forage yield. In year 2009/10 heritability values at Tallassee were observed higher than at Headland which may be due to higher mean temperature at Headland. Overall moderate to high heritability estimates for dry-matter yield were observed indicating sufficient genetic variability for further improvement.
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I whole heartedly dedicate my work to my family and especially to my wife Shikha, for their unconditional love and support.
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I. Literature Review

Species Description

The genus *Lolium* of the Poaceae family has been subdivided into eight species (Terrell, 1968) among whom only annual ryegrass (*Lolium multiflorum* Lam.) and perennial ryegrass (*L. perenne* L.) are important from an agricultural perspective. Annual ryegrass, also known as Italian ryegrass is a cool season bunch grass without rhizomes, supposed native to Italy (Beddows, 1953). The inflorescence of annual ryegrass is a solitary terminal spike, about 12 inches long with alternately edgewise arranged sessile spikelets on a central axis. The seed of ryegrass is a caryopsis, a mature ovule enclosed in two bracts lemma and palea. It has an awned lemma which distinguishes it from the awnless seed of perennial ryegrass (Jung, 1996). The number of florets per spike is thought to be controlled in a more intricate way genetically as compared to the development of awns. Thus the number of florets per spike can be a more reliable character to differentiate annual ryegrass and perennial ryegrass (Terrell, 1968).

A typical mature leaf is 2.5 to 8 inches long and 0.15 to 2.5 inches wide with glossy abaxial surface giving the plant its shiny look. Rolling of leaves occurs in young shoots while in the perennial species they are folded. As in all grasses, the stem of the plant is a culm with nodes and internodes; the height varies from 12–40 inches (Hannaway, et al., 1999).

As common name indicates annual ryegrass survives for one growing season but also behaves as a biennial in temperate regions of the world and is responsive to day length (Cooper, 1950). Ryegrass is adaptable to different kinds of soil from wet clay soils to deep sandy soils with an optimum soil pH of range 5.7 to 7.8 (Hannaway, et al., 1999). Annual ryegrass performs
best under high nitrogen content. Nitrogenous fertilizers are known to promote the turnover rate of ryegrass tillers (Hunt and Mortimer, 1982). Ryegrass has the ability to recover from mild summer drought but cannot withstand an extended period of dry weather. This is attributed to the inability of the shallow root system to maintain regular growth during drought, as commonly found in most cool season forages (Jung, 1996).

Annual ryegrass behaves as a cross-pollinated species due to gametophytic self-incompatibility, which is controlled by a pair of multi-allelic genes S and Z (Fearon, et al., 1983). It is interfertile with perennial ryegrass (L. perenne) and meadow fescue (Festuca pratensis Huds.) (Jauhar, 1975). Naturally, annual ryegrass occurs as diploid (2n = 2x = 14) in nature but tetraploids (2n = 4x = 28) can also be produced with the application of colchicine (Ahloowalia, 1967). Tetraploids have wider leaves, sturdier stems with larger inflorescences and spikelets but have lower number of tillers per plant, percentage of seed set and seed number per inflorescence. For forage production, diploids perform better under dry conditions and are more suitable for hay production (Wit, 1958). Annual ryegrass grows the best under a temperature range of 20-25° but its growth rate ceases if average daily temperature falls below 6.5°C (Hannaway, et al., 1999). Annual ryegrass has the ability to germinate over a broad range of temperatures and even at extreme diurnal variation (Young, et al., 1975). Day/Night temperatures ranging from 15/2.2° C to 35/22° C have been found ideal temperature for the germination and with the rise in temperatures germination is reduced by 30% (Nelson, et al., 1992). Even though annual ryegrass germinates at the day/night temperature of 40/20-25°C, seedlings won’t survive at such temperatures (Young, et al., 1975). Both perennial as well as annual ryegrass also have relatively high winter and early spring growth under maritime conditions (Jung, 1996) which is supported by the study that with increase in mean daily
temperature from 4.5°C to 15°C the leaf growth increases i.e. in terms of higher values of leaf extension rate (Keatinge, et al., 1980).

**Use in Agriculture**

Annual ryegrass is a short duration grass chiefly used for pasture and silage in dairy and beef cattle production. High herbage yield, forage quality, palatability, rapid seed establishment, weed suppression, tolerance to a wide range of climatic conditions, and close grazing make it an excellent cool season forage under many conditions. Out of 1.2 million acres in USA, 90 % of the acreage is in southeastern region of the USA, where it is mainly used as winter pasture (Balasko, et al., 1995); 80% of these ryegrass pastures are developed through over-seeding into the warm season perennial grasses (Hannaway, et al., 1999). Annual ryegrass is a well known grassland crop that can be intercropped with legumes and can also serve as nurse crop for establishment of legume cover crops, but only when sowed at low densities (Valenzuela and Smith, 2002). The aggressive growth of ryegrass has the potential to suppress the growth of legumes but very rarely such problems have been reported. Soil drainage, availability of nutrients, weather conditions, season, legume species, grass cultivar and intensity of grazing are deciding factors which determines the compatibility of annual ryegrass with legumes (Jung, 1996). Binary mixtures of alfalfa and ryegrass reported higher yield as compared to alfalfa alone, with early maturing diploids found to be much better than tetraploids and late maturing diploids (Sulc and Albrecht, 1996) in the mixture. Due to its rapid growth annual ryegrass is a good nitrogen scavenger and can efficiently utilize nitrogen supplied from manure and biosolids applications. Its widespread shallow fibrous root system also helps in soil conservation by reducing soil erosion (Hannaway, et al., 1999).
Forage Yield and Quality

Forage yield is the dry matter available from the pasture or rangeland on per acre basis that can be either removed by grazing animals or harvested comparable to cutting and drying hay. The most common and convenient method used to evaluate forage yield is the small-plot trial. In this method a small sample is dried to determine DM percentage, enabling to estimate the harvested DM yield per unit area (Hopkins, 2000).

Annual ryegrass is known for its high herbage yield, forage quality, palatability, rapid seed establishment, weed suppression, tolerance to a wide range of climatic conditions, and tolerance to close grazing conditions which makes it an excellent cool season forage (Jung, 1996). Due to unavailability of live forage from other sources and near impossibility of producing ample quantity of hay during winter months, beef cattle producers in the southeastern USA rely on annual ryegrass, as it provides them high quality and cost-effective option for continuous supply of forage during winter and spring months.

In the Southeastern USA, ryegrass has a short growing season with unequal partitioning of growth between winter and spring months as temperature plays a key role in growth. About 40% of the total seasonal forage dry matter is accumulated during winter months (December-February) and remaining 60% during spring (March-May). In Louisiana trials April was found to be most productive month with 30% of total dry matter. This growth pattern can be used to increase winter forage production as compared to other grasses (Redfearn, et al., 2002). A multi-location study in Louisiana was conducted to study the performance of annual ryegrass cultivars over a twelve-year period. Total forage production was not found to be stable and is highly
dependent on environmental conditions. Among twelve cultivars under trial, Marshall performed best and produced 402 kg ha\(^{-1}\) more total seasonal yield than Gulf. Mean seasonal yields for early and late season forage production ranged from 2.3 - 4.6 Mg ha\(^{-1}\) and 5.1 - 7.4 Mg ha\(^{-1}\), respectively. Marshall again outperformed every cultivar across all twelve years of evaluation and yielded greater than the mean of all other cultivars by 167 and 317 kg ha\(^{-1}\) for early and late season production, respectively, while Gulf produced 216 kg ha\(^{-1}\) and -169 kg ha\(^{-1}\) in comparison to mean production of other cultivars (Redfearn, et al., 2005).

Based on the Louisiana results it can be concluded that there has been no genetic improvement in total seasonal forage yield since cv. Marshall was released in 1980 (Arnold, et al., 1981). There has been no improvement in total seasonal forage yield. Cool season perennial forages differ from agronomic crops such as maize (\textit{Zea mays} L.) or soybean (\textit{Glycine max} (L.) Merr.) regarding the areas of agronomic and seed production. Area of agronomic utilization and seed production are same for many agronomic crops but cool season forages have geographically different area of utilization and seed production. Lack of progress might simply be the result of plant breeding efforts outside its agronomic utilization target area. Casler stated that a forage trait must be selected in the geographic area of agronomic production (Casler, et al., 2003).

Forage quality is assessed in terms of relative performance of animals when fed to herbage without any advance preparation; which is also evaluated in terms of nutrient concentration, intake potential, digestibility, palatability, anti-quality factors and partitioning of metabolized products within animal (Buxton, et al., 1996). It is also defined as the extent to which a forage has ability to produce desired results (Ball, et al., 2001). Cool season forage grasses such as annual ryegrass are known for their high forage quality as they have high
digestibility and high protein content at their vegetative stage. On average, cool season grasses have been found to have 128 g kg\(^{-1}\) higher \textit{in vivo} digestibility than warm season grasses. Generally, warm season grasses (C\(_4\) plants) have large, heavily lignified bundle sheath cells resulting in less digestibility to that of cool season grasses (C\(_3\) plants). High growing temperature conditions of warm season grasses, leads to increase in cell wall contents resulting in low digestibility as compared to cool season grasses (Minson and McLeod, 1970). Cool-season forage grasses are also known to have a high protein content during their vegetative phase, most of which is easily degradable and unable to bypass the rumen (Mullahey, et al., 1992). They have usually high Ca, P, Mg and crude protein than warm-season grasses but less than that of legumes (Buxton, et al., 1996).

Annual ryegrass, red clover (\textit{Trifolium pratense} L.), white clover (\textit{T. repens} L.) and their binary mixture sown in spring barley resulted in highest yield and efficiency of total protein from the mixture of legumes with annual ryegrass (Paza, et al., 2009). A study conducted by Beck et al. (2007) has shown that addition of annual ryegrass to small grains has led to increase in forage dry matter by 17% in comparison to small grains monoculture, resulting in increased body weight gain per hectare. Adding annual ryegrass to small grains for fodder was also found beneficial as it lead to extended grazing days per hectare, since annual ryegrass grows actively during spring months (Redfearn, et al., 2002).

Forage quality decreases as plant progresses towards reproductive maturity with rapid decline in digestibility due to lowering levels of soluble plant components, sugars and proteins, and a rise in lignin and cellulose content (Parks, et al., 1964). Dry matter (DM), metabolizable energy (ME), and NDF increases, while crude protein (CP) and \textit{in vitro} dry matter digestibility
IVDMD decreases with advancing growing season (Callow, et al., 2001). In another study, a rapid decline in nutritive value was also observed with seed head emergence, as highly significant cultivar × harvest date results were observed for NDF, IVTD and DNDF (Redfearn, et al., 2002). This suggests using late maturing annual ryegrass cultivars such as Marshall, Rio and Jackson for high quality forage in late season, but at the expense of early fall or mid-season production. On the basis of the relationship observed between forage quality and maturity it is optimal to harvest ryegrass for hay production when 10 to 20% of inflorescence heads out, since at this stage IVDMD content will not be less than 770 g kg⁻¹ DM (Redfearn, et al., 2002). Growth stages are good indicators for harvesting the ryegrass and are helpful to maintain an optimum balance between dry matter yield and quality.

Feeding immature annual ryegrass daily to steers resulted in average daily body weight gains (ADG) around 1.13 kg or even more (Lippke and Forbes, 1994). Conversely in Alabama other studies have reported low body weight gains throughout the season even with a highly digestible and proteinacious diet (Ball and Crews, 1995). In one of the studies only 70% of dry matter intake was reported, where herbage mass was not a limiting factor. This was explained to be due to severe lactic acidosis (Lippke and Warrington, 1984.) resulting in less production of bacterial cells in the rumen (Allison, et al., 1975). High supply of amino acids from proteins digested in lower tract was found to be more, than the energy supplied and thus the feedback mechanism resulted in low intake of forage. Annual ryegrass selected for high magnesium content (44% higher) was more preferred by goats, comparing to control cultivar, resulting high dry matter intake (Moseley and Griffiths, 1984).
Seed Yield and Production

Seed production for cool season grasses such as annual ryegrass is different than seed production for agronomic crops such as cereals or maize or large seeded legumes or soybean, because the area of agronomic production and seed production are disjoint. Most cool season grasses require short-days, low temperatures and moisture for flower initiation and moderate temperatures, long-days and dry period for further inflorescence development and seed maturation (Mcdonald, et al., 1996). Such environmental condition with dry summers plus supplemental irrigation makes the Pacific Northwest mainly the Oregon and Washington area the “world capital” of seed production for cool season grasses including annual ryegrass, although most of its agronomic use is in the southeastern USA. Stability of climatic conditions with supplemental irrigation and uniform maturity are highly essential for production of high quality seed (Kalton, et al., 1996).

The number of vegetative tillers converting into reproductive tillers, number of spikelets per inflorescence and number of florets per spikelet are important seed yield components. Seed yield in grasses is seriously affected by sterile florets produced due to pollination failure, abortion of developing caryopses, and shattering losses (Kalton, et al., 1996). It has been observed that only 60% of spikelets are successfully fertilized resulting in a developing embryo but approximately 50% of these abort before maturation leading to a 30% overall rate of spikelets developing into a mature caryopsis (Marshall and Ludlam, 1989). Shattering loses i.e. lack of retention of seed is mainly attributed to lack of uniform maturity in grasses (McWilliam, 1980). Shattering losses in grasses are much greater than in cereals, which can be explained by lack of domestication of grasses. Cereals on the other hand are highly domesticated and were
selected for less shattering during the process of domestication. Grasses have not evolved a uniform maturation and variation in flowering time can be found to such an extent that even the florets on the same tiller do not mature at same time (Kalton, et al., 1996). Directly or indirectly many other agronomic traits such as plant height, leaf area, dry matter yield, heading date, lodging resistance affect seed production (Griffiths, 1965).

Seed yield is a complex trait that is the outcome of interaction between multiple genetic, physiological and environmental factors. Seed yield and its components are found to have large genetic variation, which can be attributed to low selection pressure. Most breeding projects have concentrated on increasing forage yield rather than seed yield even though it is very important trait that determines the economic viability of a cultivar (Jung, 1996). Most studies have reported high heritability for the seed yield and its components in grasses. A study conducted in Norway using full sib families of meadow fescue (F. pratensis Huds.) reported phenotypic and genotypic variation for seed yield and various traits affecting it. Heritability values for seed yield components (heading date, plant height, number of fertile tillers, 1000-seed weight, panicle length, seed weight per panicle, fertility,) ranged from 0.50 to 0.80. Panicle fertility, fertile tillers, plant height and flag leaf width were found to have most effect on seed yield, which is also confirmed by other studies in which high broad sense heritability values were observed for number of spikelets/spike, number of florets/spikelet and 1000-seed weight (Fang, et al., 2004).

In annual ryegrass, Elgersma et al. (1989) reported high heritability values for seed yield components such as number of spikelets/spike, number of florets/spikelet, and 1000-seed weight while seed yield per unit area itself has low heritability. Similarly, another study conducted on annual ryegrass showed high heritability values with high additive variance for number of
spikelets/spike, spikelet length, and inflorescence number and length. However, these traits had a low genetic correlation with seed yield (Cooper, 1960).

Negative correlation between seed yield and important vegetative agronomic traits like dry matter (DM) yield, leafiness, and forage persistency is thought to be a major constraint for breeding increased seed yield in grasses. Many studies conducted in *Lolium* species have reported either negative or no genetic correlation between forage yield and seed yield. Anderson (1981) reported a negative relationship between seed yield and DM yield but the values were not large enough to matter. Conversely, increase in seed yield has been reported with increase in DM yield when plants were selected for high winter growth in annual ryegrass. With two cycles of phenotypic recurrent selection, seed yield was increased by 204 Kg ha\(^{-1}\) with a significant year × population interaction (Dhaliwal, et al., 2009) but this study was conducted in the same location as the selection. Similarly, other studies by Araujo and Coulman (2004) and Fujimoto and Susuki (1975) also observed a positive correlation between seed yield and DM yield in brome meadow grass (*Bromus riparius* Rehm.) and annual ryegrass respectively.

**Recurrent Selection**

In breeding programs, selection is the directional force that determines the genetic change in populations (Comstock, 1996). “Recurrent selection includes breeding methods that are cyclical and conducted in repetitive manner to gradually increase the frequency of favorable alleles of quantitatively inherited traits in plant populations” (Hallauer, 1985). It is the cumulative effect of many desirable alleles and their way of assembly that controls the relative expression of the desired trait under different set of environments. Recurrent selection is really advantageous as it improves a population for a certain trait without reduction in its genetic
variability and also making way for future improvement. Recurrent selection can be applied to
autogamous and allogamous species for wide range of traits and has been employed in
conjugation with other popular breeding methods like the pedigree method, in order to widen the
genetic base. Recurrent selection has been widely used in forage breeding programs since the
traits of interest such as forage yield, seed yield and other quality traits are quantitative in nature
(Nguyen and Sleper, 1983).

Every recurrent cycle has three phases: progeny development, evaluation of progenies
and recombination of superior progenies conducted in a repetitive and cyclical manner. A
recurrent selection cycle is initiated by developing progenies for evaluation. These progenies can
be half-sibs, full sibs or selfed one. Type of progeny developed is dependent on factors such as
plant species, traits under selection and relative efficiency of selection. In the evaluation phase,
progenies are then selected under certain environment(s) on the basis of a given objective of the
experiment and the best performing progenies are selected accordingly (Hallauer, 1985).

The final phase of a selection cycle is the recombination of selected progenies with the
main aim to form a population for continued selection. Under ideal conditions, gametes of
selected progenies should be equally represented in thus created population. Adequate
recombination is a must to form the new genetic recombination providing the further basis for
improvement. Hanson has reported four to five generations of recombination to be sufficient for
breaking the initial linkage blocks (Hanson, 1959). Optimum number of progenies is critical in
order to screen the genetic differences in the population. Evaluation of complex traits such as
grain yield requires extensive testing and more number of progenies so as to find the differences
among them. Number of progenies to be selected for recombination is critical and depends on a
number of factors such as population size of progenies to be tested, heritability of trait, selection intensity, effective population size and objectives of selection (Hallauer, 1985). Certain studies have reported the recombination of 30 to 45 number of individuals with selection intensity of 10% to be optimum so as to have better response to selection but still avoiding detrimental effects of inbreeding and genetic drift (Baker and Curnow, 1969). The method used for selection of progeny depends chiefly on the trait(s) to be selected. To improve a single trait, a simple truncation method is often used. However, for improving the overall agronomic performance in which number of traits are considered, sequential selection is preferred at different growth stages or delayed final harvesting may be done since all data for different traits will be available at the end (Hallauer, 1985). There are many ways and modifications to recurrent selection but on the basis of type of population improvement, recurrent selection method can be classified broadly into two categories, i.e., intra-population recurrent selection and inter-population recurrent selection.

Intra-population methods are more commonly used and easier to carry out as the primary gene action is additive in nature. In contrast, inter-population methods have been employed in cases where the quantitative traits under improvement have shown non-additive effects, mainly dominance effects.

In an intra-population recurrent selection method, variability in the response to selection can be due to limited number of cycles conducted, traits to be improved, genetic variability, type of gene action, and effectiveness to utilize those additive gene effects. Traits such as yield that have low heritability will require more number of cycles of selection as compared to traits with high heritability and effective screening tools.
Intra-population methods of recurrent selection include phenotypic recurrent selection, recurrent selection for general/specific combining ability, and S1 or S2 progeny recurrent selection with many modifications that have been developed for these methods. In phenotypic recurrent selection methods selection is based solely on the phenotype of a plant. All three phases viz. evaluation, selection and recombination are completely based on single plants; no progeny or combining ability tests are performed.

In case of half-sib recurrent selection of annual species, progeny families/individuals are randomly sampled from the population, which are then evaluated and selected for the recombination phase based on performance of their progenies in second year/season. In the third year, recombining either remnant seed from selected parents or selected progeny generates the population for next cycle; the latter case offers the opportunity to conduct within, in addition to among half-sib family selection. Among-and-within family selection can be more effective than family selection, in case the selection criterion with-in family is heritable and shows positive correlation with desired character. Among-and-within family selection also outperforms the progeny-test selection when heritability on the individual plant basis is more in comparison to heritability on family mean basis (Casler and Brummer, 2008). Full-sib selection method is similar to half-sib methodology with only difference being that full-sib families are generated from bi-parental crosses using parents from the base population or population generated by reconstitution of selected plants in previous cycle. Half-sib recurrent and full sib-recurrent selection is also practiced using a common tester for selecting the progenies. Selection of tester is very critical and it can be of broad genetic base or narrow genetic base depending on whether the tester is used for testing general combining ability (GCA) or specific combining ability (SCA) respectively.
In a recurrent selection program, selection can also be done on the basis of $S_1$ or $S_2$ performance, which is known as $S_1$ or $S_2$ progeny recurrent selection, respectively. Selfing can be done for one generation ($S_1$) or for two ($S_2$) generations depending on plant breeder’s discretion. The chief rationale behind using this approach is to increase the magnitude of additive genetic variance. Both selection methods ($S_1$ and testcross) have been useful for improving the populations under selection and certain studies have found testcross selection to be more effective than $S_1$ or $S_2$ selection (Horner, et al., 1973, Lonnquist and Lindsey, 1964). In contrast, Burton et al. (1971) reported higher gain per cycle in $S_1$ selection as compared to testcross selection. They also reported considerable evidence that different genes are selected while selecting for $S_1$, which is supported by the large enough difference in gene frequency of populations selected by the two methods. But $S_1$ and $S_2$ methods cannot be applied in highly self-incompatible crops such as annual ryegrass.

Inter-population methods involve two populations, so application of inter-population methods is limited to the crop species where heterosis can be exploited and sufficient seed set from cross pollination is not a problem e.g. maize, sunflower and sorghum. Rate of direct response is measured in terms of improvement of a cross to its parents and depends on genetic variability, differences in gene frequency, and the mean performance of the two base populations. In some cases, rate of direct response has been reported same for both inter and intra-population recurrent selection methods but the intra-population methods have applications to wider range of traits, utilizing additive variance and much more simple to conduct. Due to these factors intra-population recurrent selection methods are more popular than inter-population methods. Use of inter-population methods can aid us to study the non-additive effects and
effectiveness of selection to these effects. Inter-population methods can also be potentially used
to generate new germplasm which can be further utilized in other breeding programs.

Inter-population population improvement approach mainly includes reciprocal recurrent
selection, which is used to improve the performance of two populations simultaneously by
crossing between them. This method was originally developed by Comstock and co-workers
using half-sib approach and each population acts as tester for each other and helps in improving
two populations for their GCA and SCA (Comstock, et al., 1949). Later Hallauer and Eberhart
made modifications by using full-sibs to make it more efficient, because variance among full sibs
is one-half of the additive variance rather than one-fourth in the case of half-sibs (Hallauer and
Eberhart, 1970). Reciprocal recurrent selection programs have been developed and applied
chiefly in maize using both half-sib (Paterniani and Vencovsky, 1978) and full-sib approaches
(Marquez-Sanchez, 1982).

Except for phenotypic (= mass) selection, all methods employ progeny testing for
selecting superior genotypes for recombination, which results in an increase of both time and
cost per cycle. Since phenotypic recurrent selection does not require progeny testing, one cycle
can be completed within a year in case of annual crops. In perennial crops, the shortest time per
cycle will be determined by the first time reproductive development is initiated, which may be
several years after germination as would be the case in tree crops. Weyhrich et al. (1998)
compared seven methods of recurrent selection in the same maize population. The gain per cycle
for mass selection was less as compared to the half-sib and full-sib genotypic selection but gain
per year (0.029 Mg ha\(^{-1}\)) was almost equal. Mass selection in maize was highly cost effective as
it required only $12,123 per unit gain, in comparison to $100,250 and $190,058 per unit gain for
half-sib and full-sib genotypic selection methods respectively (Table 1.1). Furthermore, much higher return on investment for mass selection (8.25 * 10^5 Mg ha^-1 $^{-1}$) have been reported than the half-sib and full-sib methods and rest of four recurrent selection methods used in this study (Table 1.1). Similar results were obtained in another study conducted in orchardgrass (*Dactylis glomerata* L.), in which phenotypic recurrent selection for resistance to rust (*Puccinia graminis* Pers.) achieved same level of resistance per year as compared to polycross progeny test (Miller and Carlson, 1982). To enhance germplasm and to develop cultivars, recurrent selection procedures have always been considered as a good option. Any successful recurrent selection program relies on genetic variation, a sexual mode of reproduction, and the ability to produce seed on cross-pollination. Forage crops have all these qualities plus less genetic uniformity, hence recurrent selection is better suited for them than cereal crops. Many cross-pollinated species of forage crops have shown progress and responded well to the recurrent selection. Even though many of the basic principles were developed and applied for the first time on maize, they are also generally applicable to forage crops. Nevertheless, the procedures of conducting recurrent selection in many forage species differ from maize because of their perfect flower and in some instances self-incompatible nature.

The term phenotype refers here to all the physical attributes of an organism ranging from things visible, or that can be measured externally, to molecular or physiological attributes (Comstock, 1996). Application of phenotypic recurrent selection can help us to achieve goals in shorter time than genotypic recurrent selection, since the main trait for selection in forages is forage yield and selection can be performed prior to anthesis, completing one cycle in a year. As selection is based on phenotype, it is necessary to control environmental variation so as to make selection more efficient. Gardner (1961) was the first to apply a restriction on phenotypic
selection by dividing a field into small uniform plots called grids. This restriction helped to maximize the genotypic expression by reducing environmental and genotype-environment effects by providing all genotypes i.e. treatments, the same environment. A fixed number of plants are selected from \( n \) blocks with \( m \) plants per block to keep the selection intensity constant across blocks. Initially mass selection was not productive in increasing the yield in maize as it is a low heritable trait but Gardner was ultimately successful in increasing yield in maize up to 38% by applying 13 generations of selection on a grid basis.

Burton (1974) applied further restriction on mass selection by allowing only selected plants of bahiagrass (*Paspalum notatum* Flueggé) to mate in isolation in the lab during recombination phase, hence controlling both maternal and paternal gametes. This led to double the genetic gain and since recombination was done in the same year, a recurrent cycle was completed in a single year. Burton named it restricted recurrent phenotypic selection (RRPS). Application of RRPS for increasing forage yield in bahiagrass was very successful as it resulted in consistent 16.4% increase per cycle for eight RRPS cycles without any loss in genetic variation as indicated by coefficient of variation. Improved RRPS was found to be 4 times more efficient than ordinary mass selection (Burton, 1982).

Phenotypic recurrent selection has been successfully employed in grasses to improve quantitative traits. In perennial ryegrass, two cycles of phenotypic recurrent selection led to increase in the seed yield by altering the seed yield components. Population subjected to phenotypic recurrent selection had more number of reproductive tillers, high seed set and higher number of seeds per tiller in comparison to unselected population (Marshall and Wilkins, 2003).
In red clover, the inheritance of petal color was studied using phenotypic recurrent selection. Seven selection cycles were conducted which resulted in change of color from pink to purple. On scale of 2 to 9 (2-light pink, 9- purple) mean of color was shifted from 3.1 to 8.2. Narrow sense heritability was reported to be highest in cycle two and seven, which can be explained by maximum total variation in cycle two and fixation of genes for the trait approached in cycle seven respectively. Earlier selection cycles were observed to have high additive variance and ranged between 0.088 to 0.246 (Cornelius and Taylor, 1981).

Cereal rye forage yield has been effectively improved through recurrent phenotypic selection. Four cycles of visual selection resulted in an average gain of 6 to 7% in spaced plant and 0 to 3 % per cycle in seeded plot yield (Bruckner, et al., 1991). In Pensacola bahiagrass, Werner and Burton (1991) studied the effect of RRPS for individual-plant forage yield on various morphological traits such as culm number per plant, racemes per culm, height, leaf length and width, raceme length, plant diameter, plant weight, culm weight, and leaf weight. RRPS increased forage yield from cycle 9 to 16 was associated with increased mean values by 10-79% above cycle 9 for all but one plant morphological trait; plant diameter decreased by 15%.

Besides increasing forage yield, phenotypic recurrent selection has also been successfully employed to improve disease resistance and nutritive value of forages. Phenotypic recurrent selection was found to be very effective in developing resistance against bacterial wilt in alfalfa (Medicago sativa L.). Four cycles of phenotypic recurrent selection performed on two populations referred as ‘A’ and ‘B’ improved the resistance score from 3.72 to 1.38 and 4.25 to 2.63 respectively on the scale of 0 to 5 (0 = healthy and 5= dead). Similarly ten populations of red clover were subjected to six cycles of phenotypic recurrent selection for resistance to
northern anthracnose (NA) disease. Resistance to NA was significantly improved with 36% reduction in mean disease severity index (DSI) and linear across cycles. All ten populations used in the experiment were reported to be low in NA-variation but yet significant progress was realized through six cycles of selection with average of less than 24% reduction in DSI in four populations and 46% in the rest of the six populations. Average realized heritability for all ten populations was 20% and values were highest among six populations with greater improvement in DSI (Taylor, et al., 1990).

**Variance Components and Heritability**

Most agronomic traits are controlled quantitatively and genetics of these metric characters revolve around the study of their variation. Variation is expressed as variance, which is estimated as squared deviations from population mean. Fisher was the first to use term variance and describe it as square of the standard deviation (Fisher, 1918). In broad sense the observed deviation from population mean i.e. phenotypic variance ($\sigma_P^2$) is due to genotypic differences and environmental causes (any non-genotypic sources of variation) and are named as genotypic variance ($\sigma_G^2$) and environmental variance ($\sigma_E^2$) respectively, $\sigma_P^2 = \sigma_G^2 + \sigma_E^2$ (Falconer and Mackay, 1996). If a correlation exists between genotypes and environment then covariance between genotype and environment is also considered in the equation $\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + 2\text{Cov}_{GE}$ (Falconer and Mackay, 1996, Kempthorne, 1957).

Genotypic variance is furtherchiefly partitioned into three components i.e. additive variance ($\sigma_A^2$), dominance variance ($\sigma_D^2$) and interaction variance ($\sigma_I^2$) $\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2$ (Falconer and Mackay, 1996). One of the intrinsic properties of a population is its gene frequency. Magnitudes of the genetic components of variance are dependent on the frequency of the gene frequencies and so will vary from population to population. Thus estimates of genotypic
variance and its components are applicable only to the population from which they are estimated (Falconer and Mackay, 1996).

Additive variance is the most important component of genotypic variance as it determines the observable genetic properties. It is also the chief cause of resemblance between relatives and known as variance of breeding values under random mating conditions (Falconer and Mackay, 1996). Additive variance is the fixable component of the genetic variance and is generally the largest portion of the total genetic variance. Additive variance is also most easy to estimate in comparison to other components. Thus for convenience, genetic variance is mainly classified into two major components viz. additive and non-additive (Falconer and Mackay, 1996).

Phenotypic recurrent selection programs generally employed in forage breeding program relies on additive and additive x additive part of genetic variance components for improvement variance (Hallauer, 1985).

Non-additive variance consists of dominance and interaction variance. Dominance deviation arises due to dominance action among alleles and also known as deviation due to within locus interaction, while the deviation due to the interaction among genes at different loci, i.e. inter-locus interaction is known as epistatic deviation. Fisher (1918) was first to construct a gene model to study the inheritance of quantitative traits with dominance at single locus and further stated about the deviation from simple additive effects among loci in case of more than one locus and gave it a term called “epistacy”. In statistical terms the epistatic deviation is defined as the deviation of multilocus genotypic values from the additive combination of their single-locus components (Lynch and Walsh, 1998).

Based on the number of loci involved, epistatic or interaction variance can be further subdivided into two way or three way interaction respectively, which is further subdivided based
on type of interaction e.g. additive x additive variance ($V_{AD}$), additive x dominance variance ($V_{AD}$), dominance x dominance variance ($V_{DD}$). 

$$V_I = V_{AA} + V_{DD} + V_{DD} + etc$$

Such partitioning of genetic variance was first independently given by both Cockerham (1954) and Kempthorne (1954). In contrast, this notion of epistasis is argued to have a potential to make a significant contribution to additive and dominance variance and cannot be rejected outright (Cheverud and Routman, 1995).

Analysis of variance (ANOVA) was first developed by Fisher (1918) and applied to demonstrate the distribution of the additive and dominance portions considering the correlations among relatives in a randomly mating population. For estimating the parameters of genetic variance, excellent work has been published by Cockerham (1963), Comstock and Robinson (Comstock and Robinson, 1948, 1952), Gardner (1963), Matzinger (1963). In a simple context, genetic variation can be quantified from phenotypic variation by using clones or inbred lines. Variation in clones or inbred, measures the environmental variation, which then can be deducted from total phenotypic variation resulting in an estimate of genetic variation. But further estimation of components of genetic variation requires progenies with identical genetic relationships as the genetic variation is actually estimated from the covariance between sibs. To produce such groups of individuals with a common genetic background requires special mating designs (Dudley and Moll, 1969). Full sibs share half of their genes while half-sibs share one quarter of their genes, so both cases estimates one-half and one quarter of the additive variance respectively. Many different mating designs have been developed but its selection should be done according to the desired information from the experiment (Dudley and Moll, 1969). For example there are one-way, nested and different cross-classified designs but one-way design is
sufficient to detect the presence of genetic variability. For mating designs the important assumptions are that parents are randomly selected from the genetic population and experimental errors are independent (Dudley and Moll, 1969).

All designs use inbred lines to produce F₁ progeny, which is then further selfed to produce F₂ plants and which are then used as parent groups. The NC design I is a nested design analyzed in a one-way ANOVA, while NC design II and III require two way ANOVA. NC designs are mainly used for estimating dominance variance. More complex designs have been developed with the aim of estimating epistatic variances by creating additional covariance relationships that allow the estimation of additional components of variance. Most of these complex mating designs allowed estimating mainly digenic epistasis and in very few cases the trigenic epistasis. Cockerham (1954) gave a genotypic factorial model in attempt to measure the epistatic variance. Using an example of two loci and assuming a locus as a factor and its genotype as a treatment level, Cockerham was able to relate different genetic effects with other model effects. Assuming that linear term in ANOVA in a one-locus scenario represents the additive variance, the quadratic term can then be interpreted as dominance variance. The linear-by-linear interaction then is the additive-by-additive epistatic value. Similarly, the quadratic-by-quadratic interaction can be interpreted as a dominance-by-dominance epistatic effect. Linear-by-quadratic interaction represents the additive-by-dominance epistatic effect. This model by Cockerham laid the theoretical foundation for understanding epistasis. Cockerham (1956) attempted to generate additional covariance by using the parents which were at different levels of inbreeding by modifying the NC design I and II but progeny evaluated in these cases are non-inbred. Cockerham’s proposed procedure was employed by Eberhart (1961) and Silva (1975) for estimating the epistatic variance in maize populations.
In an attempt to generate more covariances of relatives, Rawlings and Cockerham (1962, 1962) developed the triallel and quadrallel analyses. Triallel and quadrallel analysis provide nine covariances among relatives and this analysis uses F-tests for the presence of epistasis and its estimation in analyses of variance. Wright (1966) used diallel and triallel analysis for estimation of epistasis in the maize variety, Krug Hi Synthetic 3, which involved nine mean squares. Kempthorne (1957) suggested a complex mating design providing 11 variances and 55 covariances and was used by Chi (1965) to estimate epistasis in the open-pollinated maize variety Reid’s Yellow dent. Estimation of epistatic variances through integration of phenotypic values and pedigree information has not provided much information. In some cases epistatic variance estimates were reported to be negative and were two times greater than their standard errors. In some cases qualitative evidence was found rather than quantitative. Most of the studies had adequate sampling and testing, and it is generally concluded that either the epistatic variation is very small or the genetic models developed are not able to handle it (Hallauer and Miranda, 1988). It is also supposed that the correlation among the coefficients of additive, dominance and epistatic variance is a major confounding factor (Hallauer and Miranda, 1988).

Epistasis, being a higher order effect can substantially affect both additive and dominance variance, depending on the gene frequency (Lynch and Walsh, 1998). Further, assuming a trait controlled by \( n \) alleles will have diallel or triallel effects of order \( n^2 \) and \( n^3 \) respectively and so on. So, interaction deviation involving high number of alleles is expected to be of very low and difficult to estimate with little contribution towards the total variance. Dudley et al. (1969) estimated both additive and non-additive genetic variances while working on autotetraploid alfalfa (Medicago sativa L.) and found non-additive genetic variance to be considerably less than the additive part. Similar results have also been reported in number of other studies (Hallauer and

In most instances populations produced are genetically heterogeneous, as they are not produced from specific mating design. In such cases a general linear model (GLM) should be applied instead of ANOVA. GLM was first introduced by Cockerham (1980) for analyzing ANOVA. ANOVA models can be expressed in GLM and thus GLM approach can be used for both complicated and simple mating designs (Seber, 1977). A typical linear model can be defined as $y = X\beta + \epsilon$, in which the observed phenotypic values and all the three genetic components are defined as the regression coefficients.

Henderson (1950) was the first to formulate the concept of mixed models. Since then mixed models have been widely used for estimating the different components of variation. Cockerham (1980) further clarified the difference between fixed and random effect models. In case of fixed effect model the researchers are more concerned with the estimation of genetic differences among founders under investigation but not the population from which founders are sampled. On the contrary, if founders are sampled randomly, it leads to a random model as the genetic effects in this case are considered to be random. In this case the genetic variance of the population from which the founders are can be inferred (Xu, 2003).

Mixed models analysis is by far the predominant methodology used for estimation of parameters as mixed model methods overcome the problems faced in ANOVA. Such analysis involves the use of different methodologies such as least squares (LS), maximum likelihood (Fang, et al., 2004) methods, restricted maximum likelihood method (REML), best linear unbiased prediction (BLUP). Unlike ANOVA these methodologies can estimate variance
components with complex pedigrees and even with unbalanced sample sizes, which is the situation encountered in many field experiments.

Cultivar development programs in most of the cross-pollinated forage crops has focused on identifying superior parents, for which understanding the quantitative genetic parameters and principles on which it is based is a must for choosing efficient selection strategy (Dudley, 1997). Heritability is defined as the proportion of phenotypic variance which is due to all genetic effects (Nyquist and Baker, 1991) which is also termed as the broad sense heritability (H). In a biometrical sense it can be defined as a measure of empirical resemblance. Since different observational units and mating designs have been used, the meaning of the heritability is understood in different ways (Xu, 2003). So, as to avoid such confusion regarding the concept of heritability, Hanson (1963) defined heritability as “the fraction of the selection differential expected to be gained when selection is practiced on a defined reference unit”. Generally heritability is expressed in two ways:

1. As a relationship between phenotypic variance ($V_p$) and genotypic variance ($V_G$) known as broad sense heritability (H), $H=V_G/V_P$. Broad sense heritability represents the proportion of phenotypic variance due to the total effect of genes in a population.

2. As a relationship between the additive variance ($V_A$) and phenotypic variance known as narrow sense heritability ($h^2$), $h^2=V_A/V_P$. Narrow sense heritability represents the proportion of the phenotypic variance due to the additive effect of genes. The narrow sense heritability is of the chief cause of resemblance between relatives and is of major importance in the breeding programs.

The above two forms of heritability can be also understood as; the broad sense heritability means that trait is heritable and is determined by the genotype. The narrow sense
heritability means trait is heritable but explains how reliably it is transmitted from parent to offspring (Udall, 2003). The concept of narrow sense heritability is important in any breeding program because it is used for predicting the progress made out of selection and recombination of best individuals. Contrarily, broad sense heritability is only more useful when whole genotypic variance can be exploited as in case of clonally propagated species.

Estimation of heritability is of importance for both plant and animal breeders as it is helpful in calculating the expected gains from any selection scheme. In case of zero heritability, there is no resemblance between parents and offspring, as a result no progress can be expected regardless of the selection intensity (Falconer and Mackay, 1996). Heritability is useful in quantifying the concept that whether the progress made from the selection can be achieved easily or not. In terms of selection, narrow sense heritability $h^2$ is a fraction of the selection differential expected to be gained when selection is practiced prior to reproduction (Nyquist and Baker, 1991)

$$\Delta G = \Delta S h^2$$

Where,

$\Delta G$ is the Expected genetic progress = $\mu_1-\mu_0$

$\mu_0$ is the Initial population mean

$\mu_1$ is the Population mean after one generation of selection

$\Delta S$ is the Selection differential = $\mu_s - \mu_0$

$\mu_s$ is the mean of selected individuals

Based on this observation plus considering other factors such as relative cost of different strategies, breeders can select the optimum strategy for their experiment under given conditions
(Holland, et al., 2003). In case of significant genotype by environment interaction, heritability estimates over all environments of a particular response to selection can be compared with heritability estimates of local environments, such information can aid in determining the optimal selection scheme (Hallauer and Miranda, 1988). Heritability estimates of different populations can also aid in selecting the best base population from which maximum gains can be realized in a given time (Holland, et al., 2003). Similarly, heritability values can also aid in selecting the required family structure by comparing heritability values of different family structures originating from same base population. An indirect selection scheme may be preferred over direct selection in case the trait of interest has low heritability but high genetic correlation with the another trait with high heritability. So, the knowledge of heritability values of different traits plus the genetic correlation estimates among different traits can aid to identify the indirect selection schemes (Hallauer and Miranda, 1988). Any germplasm selected for a particular breeding program is tested and how extensively the germplasm needs to be tested depends upon heritability values.

The number of factors associated with characteristics of the population of interest and the experiment design affect the estimate of genetic variance and heritability (Holland, et al., 2003, Nyquist and Baker, 1991). The sample size of randomly selected individuals from the original population must be large enough to represent the pattern and amount of variation present in the original population, so as yield valid estimates for the original population (Dudley and Moll, 1969, Holland, et al., 2003). While estimating the heritability, the kind of genetic variance to be used as a numerator in the formula depends on the type of selection and kind of individuals or families used in the experiment. In case of $F_1$ hybrids and clones, total genetic variation can be employed while in a random mating population, additive and additive x additive variance should
be used as numerator value. Other factors are mode of reproduction (Nyquist and Baker, 1991) selection, random drift, migration, mutation and degree of inbreeding (Holland, et al., 2003).

It is important to understand that heritability is not a property of a character but also of population and the environment, thus heritability estimates must be referred to the defined population of genotypes (Dudley and Moll, 1969) and specified population of environments. Therefore change of conditions of culture management, such as planting date, density, number of replication and years will affect the environmental variance (Hanson, 1963).

Generally, estimates of genetic variance and heritability are biased in one way or another. Bias generally occurs due to inflation of narrow sense heritability by dominance and epistatic variances (Nyquist and Baker, 1991), lack of Hardy-Weinberg equilibrium, gametic phase disequilibrium, covariance due to genotype x environment interaction among different locations (Mulder and Bijma, 2005). Ignoring the cross-classified nature of year and location factors during statistical analysis can also lead to biased estimate. Considering each year by location combination as one of the environments for sake of simplicity, leads to biased estimates because family-by-environment interaction is smaller than family-by-year, family-by-location and family-by-year-by-location variances.

So as to calculate the additive part of the total genetic variance, forage breeders have generally relied on the use of half-sib family analysis. Half-sib (HS) population structures include polycross, topcross and open-pollinated populations. Half-sib mating designs are generally used in grass breeding program: (1) For quantitative genetic studies such as estimating genetic variability and heritability (2) Recombining selected entries in a recurrent selection program; and (3) Testing the combining abilities of clones for developing synthetics (Nguyen and Sleper, 1983).
Plant breeders estimate heritability based on plot or on family mean estimation. These heritabilities are defined as the proportion of phenotypic variance among plots or family means due to family genetic effects (Holland, et al., 2003). In case of heritability on a per plot basis, the plots are the lowest unit of observation or selection used for predicting the genetic gains. Formula for estimating heritability on per plot basis:

\[ h_f^2 = \frac{\sigma_F^2}{\sigma_F^2 + \sigma_{FE}^2 + \sigma_e^2} \]

Where,

\[ \sigma_F^2 \text{ - Family Variance component} \]
\[ \sigma_{FE}^2 \text{ Family by environment variance component} \]
\[ \sigma_e^2 \text{ Experimental error variance component} \]

The family variance component (\( \sigma_F^2 \)) is the genetic variance among the half-sib families. Assuming diploid behavior, random mating population in equilibrium with no epistatic variance genetic variance and non-inbred parents the genetic variance among families half-sib can be used to estimate the additive variance, \( \sigma_F^2 = Cov(HS) = 0.25\sigma_A^2 \). In case of heritability based on family means is useful when selection among family is practiced, such as in case of families grown in replicated plots across different environments. Formula for estimating heritability estimates based on family-means:

\[ h_f^2 = \frac{\sigma_F^2}{\sigma_F^2 + \sigma_{FE}^2/e + (\sigma^2/e_T)} \]

While estimating heritability another consideration that should be taken into account is to define specified population environments to which the estimates must refer and to specify the selection unit. Selection unit is important because variance among selection units depends on whether individuals or families are evaluated (Holland, et al., 2003). In order to avoid biased
estimates multiple locations and years should be taken into consideration when genotype by
environment interaction is important (Dudley and Moll, 1969, Nyquist and Baker, 1991).

The first forage breeders to use a statistical approach for estimating genetic variances
were (Burton, 1951, Kalton, et al., 1952, McDonald, et al., 1952). They used variances from F_1
and F_2 (Burton, 1951) or from clones and S_1 (McDonald, et al., 1952) progenies to estimate the
genetic variances and broad sense heritability. While working with tall fescue (*Festuca
arundinacea* Schreb.) Burton and DeVane (1953) were first to use variance component method
to estimate heritability in a perennial forage grass. They estimated genetic variances from mean
squares of clones and error terms in the regular analysis of variance components. Newell and
Eberhart (1961) while working with switchgrass (*Panicum virgatum* L.) were first to use to split-
plot model design (Steel and Torrie, 1980) to calculate heritability using clones and progenies.
In order to calculate additive variance from the total genetic variance forage breeders have
generally employed half-sib family analysis. Forage grasses are mostly diploids or allopolyploids
which behave as disomics and so can be considered as diploids from statistical point of view
(Nguyen and Sleper, 1983). Assuming Mendelian inheritance diploid in nature and random
mating population, the covariance between half-sibs is linear function of additive genetic
variance and additive×additive type of epistatic interaction variance (Falconer and Mackay,
1996, Kempthorne, 1957). Half-sib families are generally analyzed in a randomized complete
block design with equal number of plants in all plots

Assuming no epistasis the genetic covariance between parent and offspring is one half of
the additive genetic variance of the reference population (Falconer and Mackay, 1996). The
linear regression coefficient of an offspring values on parental values is the ratio of covariance
between parent and offspring (*Cov_{po}* ) and the phenotypic variance of parents (*σ_p^2*). Twice the
value of linear regression coefficient gives the estimate of narrow sense heritability (Casler, 1982).

In a random mating population with diploid nature and genotypic frequencies in linkage equilibrium, the covariance between parent and offspring is not affected by linkage but the covariance between half-sibs is most affected (Cockerham, 1956). So, the regression method provides the more reliable estimate of narrow sense of heritability compared to the variance component method. The validation of these estimates are based on the assumptions of Mendelian inheritance, diploid behavior, no environmental correlation among relatives, population in linkage equilibrium, and relatives must be non-inbred and can be considered random members of a reference random mating population.

So, as to avoid the environmental correlation, parents and progeny should not be evaluated in same environment but they are, then genotype × environment covariance between parents and progeny should be removed in order to have unbiased heritability estimates. To avoid these potential biases, Casler (1982) suggested two methods while estimating heritability from parent-offspring relationship. The first method is regressing the progeny means from one environment, on parent value from a different environment. In order to correct differential environmental expression and the second set of parents should be grown in the same environment as that of offspring. The regression coefficient is then multiplied by the ratio of the phenotypic standard deviation of the parents as such and the phenotypic standard deviation of the parents grown in the offspring environment.

The second method concentrates on avoiding the necessary covariances from the numerator of the heritability estimate, procedure used by Dudley et al. (Dudley, et al., 1969) and Casler (1982). In this method parents and offspring are evaluated together in replicated trials
under same environments. Breeder can choose either method based on the specific objectives, so as to be free from potential genotype × environment interactions (Casler, 1982, Casler, 1982, Nguyen and Sleper, 1983).

**Heritability Examples**

Until 1940s, breeding programs especially in maize were focussed on developing inbred lines for producing highly productive hybrids. Because of ample variation in maize populations, breeders were not much concerned with the type of variation present. In 1940s and 1950s, extensive studies were done by various maize breeders for studying partitioning of variance components and heritability, (Hallauer, et al., 2010). Various researchers have relied on North Carolina mating designs or, in a few cases techniques suggested by Mather (1949). Diallel techniques were also used to determine the relative proportions of total genetic variance that were attributable to additive and non-additive effects. Variance components and heritability estimated for number of different traits in maize has been summarized by (Hallauer, et al., 2010) (Table 1.2) These estimates were average of each trait that were reported in several studies. These estimates indicate that grain yield and components of yield tend to have lower estimates \( h^2 < 0.3 \), plant structural traits have intermediate estimates \( 0.3 < h^2 < 0.5 \) and traits related to maturity have the highest estimates \( 0.5 < h^2 < 0.7 \). The magnitude of heritability indicates the complexity of a given trait. The summarized results indicate that grain yield in maize has the lowest heritability among all the traits (Table 1.2). As yield is a consequence of combined effect of many other traits it is by far more affected by the environment (Hallauer, et al., 2010).

In order to find the possible differences of genetic variability among different types of population, five population types: F₂, synthetics, open pollinated, variety crosses and composites have been compared while estimating variance components and heritability in maize (Hallauer,
Average estimate of additive variance ($\sigma_A^2$) was found to be the highest in composites while the average estimate of dominance variance $\sigma_D^2$ was reported to be higher in F2 population (Table 1.3). Overall comparing $\sigma_A^2$ with $\sigma_D^2$, the average value of $\sigma_A^2$ was greater than $\sigma_D^2$ in all the five population types. $\sigma_A^2$ was found to be 2.5 times and 1.05 times more than $\sigma_D^2$ in case of composite population and variety cross populations respectively. In general $\sigma_A^2$ was also reported considerably higher than $\sigma_D^2$ for synthetics, open-pollinated and composite populations.

In grass species, estimates of genetic variation and heritability have been reported by many researchers. Genetic variance observed in most of the agronomic traits is mainly due to additive gene action, as in most cases traits to have reported high narrow sense heritability. Even in some studies broad sense heritability estimates have been found equal to narrow sense heritability estimates (Carlson, 1966, Newell and Eberhart, 1961, Pavetti, et al., 1994, Schaaf, 1976, Vogel, et al., 1981). Heritability values estimated by parent-offspring regression methods are generally higher than heritability values calculated by variance component method (Aastveit and Aastveit, 1989, Nguyen and Sleper, 1983, Pavetti, et al., 1994, Simonsen, 1977, Vogel, et al., 1981, Vogel, et al., 1980). The cause of inflation in parent-offspring regression is considered due to rise in environmental covariance because the parents and offsprings shared the same plot (Vogel, et al., 1980). So to control the inflation, regression of offspring in one replication with parents in another replication and vice-versa would remove such bias. Vogel (1981) used regression analysis to compare both approaches: parents and offspring in the same plots and parents and offsprings in different plots, using two families of indiangrass (*Sorghum nutans* L). The mean values of narrow sense heritability for IVDMD and forage yield were 0.94 and 0.64 with parents and offsprings in same plot while in different plots the values were 0.42 and 0.28. But for highly heritable traits such as maturity and plant height, heritability values were reported
to be same in both cases. Elaborating Vogel et. al. (1980) method, Casler (1982) showed that their estimates were still biased due to some genotype\texttimes{}environment interaction.

Casler (1982) using parent-offspring technique calculated heritability estimates in reed canarygrass \textit{(Phalaris arundinacea L.)} under several environmental conditions. Dry matter yield heritability estimates were 0.19 (same replication, location, and year), -0.04 (different years and location) and 0.05 (different locations, same year). A similar trend was observed for canopy height; i.e. 0.79 (same replication, location and year), 0.48 (different years and location) and 0.52 (different locations, same year).

Estimates of heritability range widely among different traits. The values are reported to be lower for traits such as forage yield and fiber concentration which are complex in genetic control while higher for simple inherited traits such as heading date (Elgersma, 1990, Quesenberry, et al., 1978, Vogel, et al., 1981) and plant height (Berdahl and Barker, 1997, Elgersma, 1990, Kalton, et al., 1952, Kneebone, 1958, Lebsock and Kalton, 1954, Sachs and Coulman, 1983, Schaaf, et al., 1962).

In the past century, forage breeding programs have concentrated on improving the forage yield. Casler (1998, 1996) explained the basic three approaches that historically forage breeders have used for improving forage yield. The first and most common approach was to develop synthetics from the selections among the different classes, divided based on the morphological and maturity bases and later selecting directly for high forage yield among synthetics in advanced generation. Such approach was mostly adopted by private breeding programs. The second most common approach was to make bi-directional selection for the plants based on certain morphological trait. Public breeders mostly relied on this approach and resulted in release few cultivars. Leaf weight, leaf area expansion rate, mesophyll cell size and dark respiration rate
have been successful selection criteria. The third approach is the direct selection for the forage yield, which has been found to be least used among all three approaches (Alderson and Sharp, 1995, Casler, et al., 1996, Humphreys, 1997).


Improving forage quality has been under consideration but no serious steps were taken until development of analytical chemistry and rumen fermentation technology in mid-twentieth century which aided forage breeders in selection (Casler and Vogel, 1999). Breeding for forage quality also got boost with the advent of low cost technology such as near infrared reflectance spectroscopy (NIRS) helpful in predicting NDF, ADF and CP values. Studies evaluating the inheritance of quality traits in forages crops depict low to moderate heritability for most quality traits (Bughrara, et al., 1991, Casler, et al., 1987, Marum, et al., 1979, Pavetti, et al., 1994, Soh, et al., 1984, Stratton, et al., 1979). In perennial grasses based on single plants, realized heritability values for forage quality traits lie between 0.2-0.3 (Casler and Vogel, 1999).
In meadow bromegrass de Araújo et al. (2002) under a single harvest management system and open-pollination, reported narrow-sense heritability estimates for DMY, CP, and NDF were $0.33 \pm 0.21$, $0.08 \pm 0.29$, and $0.21 \pm 0.26$, respectively, in 48 half-sib families. In another study on smooth bromegrass (*Bromus inermis* Leyss.) by Tan et al. (1978), narrow-sense heritability estimates were reported $0.46$ and $0.19$ for ADF of the leaf-blade and other plant fractions respectively but were very low for ADF and CP of whole forages $0.03-0.08$. The relative magnitude of the variances due to general and specific combining ability suggest that majority of genetic variance appeared to be non-additive. Similar results are reported by Annicchiarico and Romani (2005) in Mediterranean tall fescue (*Festuca arundinacea* Schreb.) . Study reported low additive genetic variation for lower NDF and ADF with large, genotype and harvest interaction. Some other studies present contrary results that suggest higher additive variance for quality traits (Ross, et al., 1970, Sleper and Drolsom, 1974). Pavetti et al. (1994) have also recorded very low narrow sense heritability values for IVDM, NDF, ADF while Nguyen et al. (1983) and Bhughara et al. (1991) reported moderate values. Vogel (1981) reported average narrow sense heritability were $0.43$, $0.42$, and $0.50$, for forage yield, IVDMD, and protein, respectively for two populations in indiangrass (*Sorghastrum nutans* (L.) Nash.). Literature suggests additive genetic variance was mostly larger than specific genetic variance, and tended to be small to moderate for quality traits and moderate to high for DM yield.

Forages breeding efforts have concentrated on the vegetative growth quality, which is the economic part while ignoring seed yield. Seed yield of forage cultivars is generally low and unstable (Elgersma, 1990, Griffiths, 1965) but in terms of seed production economics seed production has become important as the success of a new cultivar depends on it. To select for high and stable seed production plant breeders have estimated broad sense heritability for seed
yield components (Bean, 1972, Bugge, 1987, Hayward, 1983, Rognli, 1987). Bugge reported heritability values of 0.92, 0.83, 0.71, 0.64 and 0.49 for ear length, numbers of spikelets per ear florets per spikelet, seed yield per plant and fertile tiller number respectively. Elgersma (1990) studied heritability values of 17 traits in space-planted perennial ryegrass. Most traits studied were seed yield components as well as maturity. Except flag leaf width, first date of anthesis, ear length and number of spikelets per ear, the narrow sense heritability estimates from parent-offspring regression and variance components among half-sib families were reported to be low.

In spite of almost century long efforts in forage breeding, the improvement in forage yield has been very small, especially in comparison to grain yield in cereals. According to an estimate, gains made in forage yield are less than 10% that of gains made in cereal crops (Casler and Brummer, 2008) Figure 1.01. A similar report has been published by Humphreys (1997) according to which on average, the overall genetic gain is of only 4% decade\(^{-1}\) compared to 13.5% decade\(^{-1}\) for grain crops. Casler (1998, 2001) and Humphreys (1997) gave different reasons for this yield lag between forages in comparison to cereal crops. i) Concentration on number of different economically important traits among which most of them are not correlated or negatively correlated; ii) Lack of harvest index trait so as to increase dry matter partitioning more into the economic product; iii) Longer breeding cycles for forage crops especially in case of perennials; and iv) Inability to exploit heterosis at commercial level.

Slow rate of improvement in the forage yield can also be attributed to lack of direct selection (Asay, et al., 1968, Carlson, 1966, Hovin, et al., 1976) for it, as per se in many cases, since yield is not easy to measure (Brummer, 2005). In addition to this, another reason for lack of progress in forage yield might be the use of post synthesis selection procedure which is inefficient as it makes make little use of additive genetic variance within half-sib or full-sib
families compared with recurrent selection (Casler and Brummer, 2008). In past century even
though the forage breeders might not have been under much pressure from forage producers to
create cultivars with higher forage yield but recent focus on forage crops as a bioenergy crops
has certainly shifted more attention towards improving biomass yield (Perlack, 2005).

As literature stresses, the lack of improvement in forage yield may be due to less use of
direct selection approach, direct phenotypic recurrent selection was employed to increased winter
dry matter productivity in annual ryegrass (Dhaliwal, 2009). Due to low availability of live
forage during early winters, beef and cattle producers rely on stored forage to meet nutritional
requirements of animals. So, increasing early winter productivity would be a great boost for the
beef industry. In order to increase early winter productivity, plants were evaluated and selected
on the basis of 750 growing degree days (GDD) post transplanting (Dhaliwal, 2009). The
recurrent selection project for improved winter dry matter productivity is currently in its sixth
cycle. Commonly as in forage breeding programs, no assessment of heritable variation was made
before the start of the recurrent selection program. Breeders of cross-pollinated non-domesticated
species generally assume the sufficient genetic variation for the traits of interest, an assumption
validated by success. Yet it is of interest to assess not only for gain from selection but also to
investigate phenotypic and genetic variances and covariances in base and selected populations.
So the objective of the current study is to measure genetic variation and heritability for dry
matter yield in three populations of half-sib families representing cycles/populations 0, 1, and 2.
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Table 1.1: Comparison of six recurrent selection methods to improve grain yield in the BS11 maize population. Source: Weyhrich et al., (1998).

<table>
<thead>
<tr>
<th>Selection method</th>
<th>Gain per cycle Mg ha$^{-1}$</th>
<th>Gain year$^{-1}$ Mg ha$^{-1}$</th>
<th>Ave. cost cycle$^{-1}$ $$$</th>
<th>Ave. cost year$^{-1}$ $$$</th>
<th>Cost unit$^{-1}$ of gain$\dagger$ $$$</th>
<th>Time to achieve one unit of gain$\ddagger$ Yr.</th>
<th>Return on investment$\S$ Mg ha$^{-1}$ $\S^{-1} \times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-sib</td>
<td>0.067</td>
<td>0.033</td>
<td>6 700</td>
<td>3 350</td>
<td>$100 250$</td>
<td>30</td>
<td>1.00</td>
</tr>
<tr>
<td>Half-sib</td>
<td>0.075</td>
<td>0.025</td>
<td>14 300</td>
<td>4 767</td>
<td>$190 058$</td>
<td>40</td>
<td>0.53</td>
</tr>
<tr>
<td>Mass</td>
<td>0.029</td>
<td>0.029</td>
<td>350</td>
<td>350</td>
<td>$12 123$</td>
<td>35</td>
<td>8.25</td>
</tr>
<tr>
<td>Modified ear-to-row</td>
<td>0.172</td>
<td>0.086</td>
<td>6 650</td>
<td>3 325</td>
<td>$38 721$</td>
<td>12</td>
<td>2.58</td>
</tr>
<tr>
<td>Reciprocal full-sib</td>
<td>0.124</td>
<td>0.062</td>
<td>12 100</td>
<td>6 050</td>
<td>$97 213$</td>
<td>16</td>
<td>1.03</td>
</tr>
<tr>
<td>S-progeny</td>
<td>0.091</td>
<td>0.046</td>
<td>7 300</td>
<td>3 650</td>
<td>$79 688$</td>
<td>22</td>
<td>1.25</td>
</tr>
<tr>
<td>S$_t$-progeny</td>
<td>0.212</td>
<td>0.071</td>
<td>10 300</td>
<td>3 433</td>
<td>$48 530$</td>
<td>14</td>
<td>2.06</td>
</tr>
</tbody>
</table>

$\dagger$ Calculated by taking cost cycle$^{-1}$ divided by the number of years required to complete one cycle.
$\ddagger$ 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$^{-1}$.
$\S$ Calculated as the inverse of gain cycle$^{-1}$ multiplied by the number of years required per cycle.
$\S$ Calculated by taking gain cycle$^{-1}$ divided by the total cost cycle$^{-1}$.
$\S$ 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.
Table 1.2: Distribution of heritability estimates for 16 traits in maize. Source: (Hallauer, et al., 2010)

<table>
<thead>
<tr>
<th>Heritability (%)</th>
<th>Traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\hat{h}^2 &gt; 70$</td>
<td>Percent oil, number of tillers</td>
</tr>
<tr>
<td>$50 &lt; \hat{h}^2 &lt; 70$</td>
<td>Plant height, ear height, kernel-row number, days to flower, grain moisture</td>
</tr>
<tr>
<td>$30 &lt; \hat{h}^2 &lt; 50$</td>
<td>Number of ears, ear length, ear diameter, kernel weight, husk extension, husk score, cob diameter</td>
</tr>
<tr>
<td>$\hat{h}^2 &lt; 30$</td>
<td>Yield and kernel depth</td>
</tr>
</tbody>
</table>
Table 1.3: Summary of estimates of additive dominance and genetic components of variance, ratio of dominance variance to additive variance, and heritability estimates for yield in five types of maize populations. Source: (Hallauer, et al., 2010)

<table>
<thead>
<tr>
<th>Type of populations</th>
<th>$\hat{\sigma}_A^2$</th>
<th>SE($\hat{\sigma}_A^2$)</th>
<th>$\hat{\sigma}_D^2$</th>
<th>SE($\hat{\sigma}_D^2$)</th>
<th>$\hat{\sigma}_D^2/\hat{\sigma}_A^2$</th>
<th>$\hat{h}^2$</th>
<th>$\hat{\sigma}_A^2/\hat{\sigma}_D^2$</th>
<th>No. of reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2</td>
<td>585.1</td>
<td>338.5</td>
<td>451.0</td>
<td>593.0</td>
<td>1.0022</td>
<td>24.4</td>
<td>1.30</td>
<td>24</td>
</tr>
<tr>
<td>Synthetics</td>
<td>225.9</td>
<td>59.3</td>
<td>128.6</td>
<td>83.4</td>
<td>0.8255</td>
<td>22.9</td>
<td>1.76</td>
<td>15</td>
</tr>
<tr>
<td>Open pollinated</td>
<td>503.8</td>
<td>178.9</td>
<td>245.8</td>
<td>320.8</td>
<td>0.7619</td>
<td>18.9</td>
<td>2.16</td>
<td>37</td>
</tr>
<tr>
<td>Variety crosses</td>
<td>306.2</td>
<td>139.2</td>
<td>292.2</td>
<td>32.0</td>
<td>1.3854</td>
<td>13.4</td>
<td>1.05</td>
<td>13</td>
</tr>
<tr>
<td>Composites</td>
<td>721.9</td>
<td>432.0</td>
<td>281.8</td>
<td>—</td>
<td>1.3335</td>
<td>13.8</td>
<td>2.56</td>
<td>10</td>
</tr>
<tr>
<td>Average</td>
<td>468.6</td>
<td>229.6</td>
<td>279.9</td>
<td>257.3</td>
<td>0.9377</td>
<td>18.7</td>
<td>1.76</td>
<td>—</td>
</tr>
<tr>
<td>No. of estimates</td>
<td>99</td>
<td>55</td>
<td>82</td>
<td>41</td>
<td>72</td>
<td>43</td>
<td>82</td>
<td>99</td>
</tr>
</tbody>
</table>

* Ratio is for the average estimates of $\hat{\sigma}_A^2$ and $\hat{\sigma}_D^2$
Figure 1.1: Comparison of genetic gains made in forage yield to the genetic gains made in cereal grains. Source: (Conaghan and Casler, 2010)
II. ESTIMATING GENETIC VARIATION AND HERITABILITY FOR DRY MATTER YIELD IN THREE POPULATIONS OF ANNUAL RYEGRASS

Abstract

Annual ryegrass, is a cool season bunch grass known to have originated in Italy and belongs to family Poaceae. Annual ryegrass is a short duration grass chiefly used for pasture and silage in dairy and beef cattle production. Due to low availability of live forage during early winters, beef and cattle producers rely on stored forage to meet nutritional requirements of animals, so increasing early winter productivity would be a great boost for the beef industry. In order to increase early winter productivity, plants recurrent selection project for improved winter dry matter productivity was initiated in which plants were evaluated and selected on the basis of 750 GDD post transplanting. As is common practice in forage breeding programs for cross-pollinated non-domesticated species simply assume that there is genetic variation for the traits of interest and no assessment of heritable variation was made before the start of the recurrent selection program. Yet it is of interest to investigate phenotypic and genetic variances and covariances in base and selected populations. To measure genetic variation and heritability for dry matter yield three half-sib populations, representing three cycles of recurrent selection were selected. In year 2008/09 the trial was sward simulated and conducted only at one location, at the Plant Breeding Unit (PBU) of the Alabama Agricultural Experiment Station’s E.V. Smith Research Center in Tallassee, Alabama while in year 2009/10 the spaced planted trial was conducted at two locations in Alabama; PBU and the Alabama Agricultural Experiment Station’s
Wiregrass Research and Extension Center at Headland (WGS). The experimental design for each trial was a randomized complete block \( (r = 2) \) with a split-split plot randomization restriction (SSP). Three harvest schemes were employed. Two were based on accumulated thermal degree-days (GDD) and the third based on heading date. Maximum likelihood methods were used to calculate variances and covariances, which further used to estimate heritability for dry matter yield. First and second cut data for first and second harvest scheme enabled us to evaluate genetic variation for productivity under autumn/winter conditions. The third harvest scheme at heading enabled us to evaluate the effect of maturity differences on genetic variation for yield. In year 2009/10 only two cuttings were done. First harvest was done at 500 GDD and second harvest was done at maturity. Considerable genetic variation among three populations was observed. Generally the heritability values have been observed higher for year 2008/09 than 2009/10, for corresponding harvests. In both years the heritability values decreased in most of the cases with the each subsequent cycle but differences among the values were not significant, since the standard error values overlapped. Similarly heritability within harvest scheme also decreased with subsequent cuts within each population, but differences among values were also not significant. The trend for the values within harvest scheme can be explained due to increase in temperature in each subsequent cut, since the populations were produced from the selection for high winter forage yield. In year 2009/10 heritability values at Tallassee were observed higher than at Headland which may be due to higher mean temperature at Headland. Overall moderate to high heritability estimates for dry-matter yield were observed indicating sufficient genetic variability for further improvement.
INTRODUCTION

Annual ryegrass (*Lolium multiflorum* Lam.) is a cool season bunch grass, belongs to family Poaceae and is supposed to have originated in Italy (Beddows, 1953). It is a diploid in nature with 2x=2n=14 chromosomes but tetraploids can also be produced with colchicine application (Ahloowalia, 1967). Annual ryegrass survives for one growing season but also behaves as a biennial in temperate regions and is responsive to day length (Cooper, 1950). Ryegrass is adaptable to broad range of soils from wet clay soils to deep sandy soils with an optimum soil pH of range 5.7 to 7.8 and performs best under high nitrogen fertilization (Hannaway, et al., 1999). Both perennial as well as annual ryegrass also have relatively high winter and early spring growth under maritime conditions (Jung, 1996) and with increase in mean daily temperature from 4.5°C to 15°C, the leaf growth increases (leaf extension rate) (Keatinge, et al., 1980).

Annual ryegrass is a short duration grass, chiefly used for pasture and silage in dairy and beef cattle production. Annual ryegrass is highly valued for forage/livestock systems due to its high herbage yield, forage quality, palatability; rapid seed establishment, weed suppression and close grazing, makes it an excellent cool season forage under many conditions (Hannaway, et al., 1999). The total area of annual ryegrass in USA is around 1.2 million ha, of which 90% is located in southeastern states and has become important component in winter forage-livestock systems (Balasko, et al., 1995) but its major seed production comes from Pacific Northwest mainly the Oregon and Washington. Generally cool season grasses require short-days, low temperatures and moisture for flower initiation and moderate temperatures, long-days and dry
period for further inflorescence development and seed maturation (Mcdonald, et al., 1996). Stability of climatic conditions with supplemental irrigation and uniform maturity are highly essential for production of high quality seed (Kalton, et al., 1996). Such environmental condition with dry summers plus supplemental irrigation makes the Pacific Northwestern states suitable for the seed production for cool season grasses including annual rye grass. So seed production for cool season grasses is different in comparisons to cereals because area of agronomic utilization and seed production are geographically disjoint. Lack of progress might simply be the result of plant breeding efforts outside its agronomic utilization target area. Casler stated that a forage trait must be selected in the geographic area of agronomic production (2003).

Due to unavailability of live forage from other sources and the almost impossibility to produce ample quantity of hay during the winter months, beef cattle producers in the Southeastern USA rely on annual rye grass, as it provides them high quality and cost-effective option with continuous supply of forage during winter and spring months. In the Southeastern USA, rye grass has a short growing season with unequal partitioning of growth between winter and spring months as temperature plays a key role in growth. About 40% of the total seasonal forage dry matter is accumulated during winter months (December-February) and remaining 60% during spring (March-May). In Louisiana trials, April was found to be the most productive month with 30% of total dry matter. This growth pattern can be used to increase winter forage production as compared to other grasses (Redfearn, et al., 2002).

Due to low availability of live forage during early winters, beef cattle producers rely on stored forage to meet nutritional requirements of animals, so increasing early winter productivity would be a great boost for the beef industry. In order to increase early winter productivity, plants
were evaluated and selected on the basis of 750 growing degree days (GDD) post transplanting, which is around mid January to early February. The recurrent selection project for improved winter dry matter productivity is currently in its sixth cycle. In each selection cycle, 1200-1600 plants were established in an evaluation nursery at the Plant Breeding Unit in late October of each year. When the target GDD was reached, all plants were cut at 5-cm height, bagged, dried, and weighed to determine dry matter yield. The entire nursery was subdivided into 25-plant blocks and the highest-yielding plant selected from each block, which amounted to a selection intensity of 4%. Selected plants were removed from the field, transferred to the greenhouse for 2-3 weeks to establish a good root system and then transplanted to an isolation nursery. Plants were allowed to cross-pollinate and seed harvested as each plant matured. This bulked seed was then used to establish the next cycle. For further detailed information regarding recurrent selection program refer to (Dhaliwal, 2009).

As it is a common practice in forage breeding programs, no assessment of heritable variation was made before the start of the recurrent selection program. Breeders of cross-pollinated non-domesticated species generally assume sufficient genetic variation for the traits of interest, an assumption validated by success. Yet, it is of interest to assess not only for gain from selection but also to investigate phenotypic and genetic variances and covariances in base and selected populations. So the objective of the study is to measure genetic variation and heritability for dry matter yield in three populations of half-sib families representing cycles/populations 0, 1, and 2.
MATERIALS AND METHODS

Source/selection of Half-sib families

Three half-sib populations, i.e., base population (C0), dry matter yield population (C2), and a population from randomly selected plant in C2-Random considered as C1, were evaluated. Accidentally the seed of C1 was lost, so plants from C2-Random were used to generate seed for C1. Since no selection was performed in the C2-random population it can be considered to have same genetic composition as C1. Each population was then increased in a replicated seed nursery with four individual isolation blocks per population. In June 2008, seed was harvested from 24 random plants per isolation block containing approximately 200 plants for a total of 96 entries per population, where each selected plant represents a half-sib family. Plants were individually harvested, placed in paper bags, threshed manually, and then fine cleaned with a South Dakota blower, thus preserving the genetic identity. In year 2008/09 the trial was conducted only at one location, at the Plant Breeding Unit (PBU) of the Alabama Agricultural Experiment Station’s E.V. Smith Research Center in Tallassee, Alabama while in year 2009/10 the trial was conducted at two locations in Alabama; PBU and the Alabama Agricultural Experiment Station’s Wiregrass Research and Extension Center at Headland (WGS).

Experiment Design

There were actually three trials conducted in parallel based on harvest schemes. The experimental design for each trial was a randomized complete block (r = 2) with a split-split plot randomization restriction (SSP). The main plot was the population with levels C0, C1 and C2-
dry matter. The subplot is the seed increase block \((b = 4)\), and the sub-sub plot and individual row plot. Plant breeders refer to this variant of the SSP as a blocks in reps design. It is commonly employed to reduce environmental variation when a large number of families have to be evaluated. The sub plot dimensions were 2 tiers x 12 rows or 3.30 x 3.60 m - a 1.5 m alley separated the tiers and provides access for seeding harvesting operations- thus making it nearly square, thereby reducing the average distance between two randomly selected points. In mid-October of year 2008/09, one gram of seed of each half-sib family was seeded manually on 90 cm rows spaced 30 cm apart to provide some competition approaching a solid seeded sward while preserving genetic identity.

In year 2009/10 plants were spaced planted. Seedlings were germinated and established in greenhouse using conetainers (Stuewe&Sons, Inc., Tangent, OR) filled with 1:1 mixture of PRO mix peat-based growing medium: sand. Seedlings were thinned to single plant per conetainer, approximately two weeks after seeding and were grown until transplanting to the field. Retaining the same experiment design, plants were spaced planted at 60 cm apart on 300 cm row plot, each plot consisting 5 plants of each half-sib family and plots were spaced at 60 cm each. In both years seeding and planting were done in mid-October and the trial area was prepared 3-4 weeks before Seeding/transplanting and treated with standard rate glyphosate 2-days prior to Seeding/transplanting so as to create a stale seedbed.

**Harvest Scheme**

In 2008/09, three harvest schemes were employed. Two were based on accumulated growing degree-days (GDD) and the third was based on heading date. Plots for the first scheme were harvested every 500 GDD, allowing an extra 250 GDD at first cut for emergence and stand establishment. First and second cut data enabled us to evaluate genetic variation for productivity
under autumn/winter conditions. Under the second scheme, plots were harvested every 1000 GDD, again allowing an extra 250 GDD for emergence and establishment. Thus every other harvest date for the first two schemes coincided. The third harvest scheme at heading enabled us to evaluate the effect of maturity differences on genetic variation for yield.

Plots were cut with electric hedge clippers at a uniform height of 5-cm using a small piece of wood as a guide along which to slide the clipper. No green matter yield data was taken. After harvest, plant materials were placed in cotton bags, and dried to consistent weight for dry matter yield determination. In year 2009/10 only two cuttings were done. First harvest was done at 500 GDD, allowing an extra 250 GDD and second harvest was done at maturity. Spaced plants within plots were individually harvested at a uniform height of 5-cm and bagged but the mean value of plants within a plot was used for data analysis. In both years forage samples taken from each plot were dried to constant weight in a forced-air oven at 60°C to determine dry weight.

Data Analysis

Estimates of the narrow-sense heritability were computed based on the genetic components of variation among half-sib families. The restricted maximum likelihood method was used to calculate variances, and covariances, which were further used to estimate heritability for dry matter yield in three populations under different harvesting schemes. Data were analyzed separately for season 2008/09 and 2009/10 and for each year analysis was done by scheme harvest and population. A completely random model was used for data analyses.

\[ Y_{jkl} = \mu + B_j + F_k + BF_{jk} + e_{jkl}, \]

where,

\( Y_{jkl} \) is the lth observation of the jth block within the kth half sib family;
\( \mu \) is the overall mean;

\( B_j \) is the random j'th block effect (j = 1,2);

\( S_k \) is the random k'th subplot effect (k = 1,2,3,4)

\( F_l \) is the random l'th family effect \((l = 1 \ldots 96)(101 \ldots 196)(201 \ldots 296)\);

\( BF_{jk} \) is the random kth family by jth block effect

\( e_{jklm} \) is the error term

Narrow sense heritability was estimated based on half-sib family mean basis averaged over replications and individual within plots and formula used was (Holland, et al., 2003, Nyquist, 1991)

\[ h^2 = \frac{\sigma_{HS}^2}{\sigma_{HS}^2 + \sigma_b^2/2 + \sigma_s^2/4 + \sigma_{bf}^2/2 + \sigma_e^2/8}, \]

where,

\( \sigma_{HS}^2 \) is the Variance among to half sib families;

\( \sigma_b^2 \) is the Variance due to blocks;

\( \sigma_s^2 \) is the Variance due to subplots;

\( \sigma_{bf}^2 \) is the Variance due to block \( \times \) Family;

\( \sigma_e^2 \) is the Error variance;

The Taylor series/delta method was used to estimate the standard errors for the heritability. All data analyses were performed using SAS® 9.2 PROC MIXED (SAS Institute, Inc., Cary, NC).
RESULTS

Harvest Scheme 1

In first scheme the plots were harvested after every 500 GDD and four harvests were done under this harvest scheme in season 2008/09 but only one harvest in 2009/10. In 2008/09 there was considerable variation in forage yield, and moderate to high heritability values were observed (Table 2.1). For the first cut, C0 had much higher additive variance out of the three populations while C1 and C2 had nearly same additive variance (Table 2.1). C0 had the highest heritability 0.57±0.26 followed by C1 0.53±0.13 and C2 0.33±0.27 (Fig 2.1). In second and third cut highest additive variance was also observed in C0 among three populations and decreased with each subsequently cycle (Table 2.1). Heritability values also followed the similar trend for cut two and cut three. Heritability values for second cut for C0, C1 and C2 were 0.69±0.09, 0.39±0.10 and 0.29±0.17 respectively (Fig 2.1). For the third cut, heritability values for three populations were 0.44±0.03, 0.36±0.20 and 0.11±0.16 (Fig 2.1). In case of fourth cut, C0 also had the highest additive variance but with high standard error while C1 and C2 had nearly similar estimated values for additive variance. Since C0 had high standard error, the difference for additive variance among three populations can be neglected (Fig. 2.2) (Table 2.1). Heritability values for the fourth cut were also nearly equal for three populations, 0.31±0.16 for C0, 0.25±0.19 for C1 and 0.31±0.17 for C2 (Fig 2.1).

In year 2009/10 only two cuts were accomplished but the study was conducted at two locations. Additive variance for all three populations was nearly equal and no particular trend for
additive variance among populations was observed at both locations. Heritability estimates for first cut at PBU were 0.43±0.16, 0.50±0.13 and 0.41±0.15 for C0, C1 and C2 respectively while at WGS estimates were 0.29±0.17, 0.29±0.18 and 0.37±0.24 (Fig. 2.3).

Harvest Scheme 2

In harvest scheme two, plots were harvested every 1000 GDD, with additional 250 GDD for emergence and establishment. The scheme was only employed in year 2008/09, so estimates were reported only for one season. For both cuts under this harvesting scheme, additive variance was observed highest for the C0 while C1 and C2 had nearly equal estimated additive variance. Heritability estimates for both cuts were high to moderate under this harvest scheme and higher than the estimates under harvest scheme one (Table 2.2). Estimates for C0, C1 and C2 for cut one were 0.84±0.04, 0.51±0.12 and 0 respectively (Fig 2.3). For cut two, estimates were 0.74±0.07, 0.61±0.10 and 0.30±0.17 (Fig 2.3). Comparing both cuts under this harvest scheme, both harvests performed equally in terms of additive variation. This harvest scheme also coincided with the maximum growth period of the crop.

Since in harvest scheme one the harvest was done after every 500 GDD and in case of harvest scheme two the harvest was done after every 1000 GDD, thus every other harvest date for the first two schemes coincided. So the yield data from cut one and two under harvest scheme one were cumulated per plot and were analyzed to compare the results with cut one under harvest scheme two. Heritability values from the cumulative yield data also resulted high to moderate heritability values, close to the heritability values from cut one under harvest scheme two (Table 2.2). Cycle C0 and C1 had comparable heritability values in both cases while heritability values
for C2 were 0 and 0.24±0.17 for cut one harvest scheme two and cumulative yield data respectively (Table 2.2).

Similarly yield data from cut three and four under harvest scheme one were cumulated, analyzed and were compared with results from cut two under harvest scheme two. Heritability values from the cumulative yield data were in the range of moderate to low and were also observed lower than cut two of harvest scheme two, except for C2 cycle (Table 2.2).

Harvest Scheme 3

In case of harvest scheme three, the plants were harvested with onset of heading. Two cuts were done under this harvest scheme in year 2008/09 while only one cut was done in year 2009/10. In third harvest scheme, for year 2008/09 moderate heritability values were observed while in year 2009/10 low heritability values were observed (Table 2.3). In 2008/09 estimated heritability values for cut one were 0.51±0.026, 0.32±0.17 and 0.49±0.13 for C0, C1 and C2 respectively while for cut two the values were 0.47±0.13, 0.39±0.16 and 0.53±0.13 (Fig. 2.3). In year 2008/09 first cut was observed to have higher additive variance then second cut under this harvest scheme but the trend in both cuts was similar.

In year 2009/10 only one cut was conducted but at two locations. Estimates at PBU were 0.29±0.17, 0.23±0.189 and 0 for C0, C1 and C2 respectively (Fig. 2.3), while heritability estimates for cut two at WGS were 0.11±0.16 for C0 and zero for both C1 and C2 (Fig. 2.3). At both locations, for both cuts no particular trend was observed for additive variance among populations, and standard errors were overlapping (Table 2.3).
Discussion

Considerable genetic variation among three populations was observed. Year 2008/09 was a sward simulation while year 2009/10 was spaced planted. Generally, higher heritability values were observed for year 2008/09 than 2009/10, for corresponding harvests. In past, studies have been conducted in forage grasses to estimate the heritability for different traits, under both spaced planted and sward conditions and to study the genetic correlation between them. The literature has been ambiguous about the genetic correlation between spaced planted and sward conditions, especially for the trait like forage yield. But most of the studies have reported higher or equal heritability values for spaced planting in comparison to sward (Annicchiarico, 2006, England, 1975, Waldron, et al., 2008). In annual ryegrass, England (1975) tested both spaced planted and sward. Heritability values for forage yield were reported 0.24-0.59 for spaced planted plots while 0.35-0.55 for sward. Contrary to most studies in forage grasses, England (1975) also reported high genetic correlations, +0.7 to +0.8 for forage yield between spaced planted rows and sward. In present study, heritability estimates were also observed in similar range but heritability values for spaced plant plots were generally less than sward plots.

In both years the heritability values decreased in most of the cases with the each subsequent cycle but differences among the values were not significant, since the standard error values overlapped. Similarly heritability within harvest scheme also decreased with subsequent cuts within each population, but differences among values were also not significant. The trend for the values within harvest scheme can be explained due to increase in temperature in each subsequent cut, since the populations were produced from the selection for high winter forage yield. In year 2009/10 heritability values at Tallassee were observed higher than at Headland which may be due to higher mean temperature at Headland.
Annual ryegrass is mainly a diploid (2n=2x=14) in nature but autotetraploid (2n=4x=28) can be produced on treating with colchicine. Tetraploids have wider leaves, sturdier stems with larger inflorescences and spikelets but have lower number of tillers per plant, percentage of seed set and seed number per inflorescence. Tetraploids are comparable with diploids in terms of dry matter production. To generate the seed of base population C0 for the recurrent selection program, two tetraploid cultivars may have been included with four diploid cultivars and were allowed to randomly mate for two years. A recent detailed analysis completed by (Poudel & van Santen, 2013, personal communication) indicated the presence of around 10% of tetraploid plants in C0 while none were found among 400 C2 plants calculated. No triploid individuals were found in either populations.

Increase in polyploidy level complicates the partitioning of genetic variance due to higher intra-allelic (dominance) and inter-allelic (epistasis) interactions. So existence of tetraploids in base population may have resulted in higher non-additive variance and thus upward bias in heritability estimates (Lynch and Walsh, 1998, Nyquist, 1991). But since epistasis, being a higher order effect; assuming a trait controlled by n alleles will have diallel or triallel effects of order n² and n³ respectively and so on. So, non-additive interactions involving high number of alleles is expected to be very low and thus can be neglected. Dudley et al. (1969) estimated both additive and non-additive genetic variances while working on autotetraploid alfalfa (Medicago sativa L.) and found non-additive genetic variance to be considerably less than the additive part. Similar results have also been reported in number of other studies (Hallauer and Wright, 1967, Isik, et al., 2003, Laurie, et al., 2004).

Since annual ryegrass is highly cross-pollinated crop due to gametophytic self-incompatibility, chances of crossing between diploids and tetraploids is high, resulting in the
formation of triploids. This would have lead to decrease in the proportion of tetraploids with every selection cycle. So it can be concluded that the bias in the heritability estimates due to tetraploids in the population can be neglected.

**Conclusion**

In conclusion moderate to high heritability estimates for dry-matter yield indicate sufficient genetic variability for further improvement. Based on our experiments, it can be possible to change the dry matter yield in annual ryegrass in a few generations of selection under high selection intensities. Breeding schemes such as phenotypic recurrent selection can be suitable and cost effective breeding method, which allow high recombination, combining the effective factors required to achieve desired dry matter yield in annual ryegrass.
References


Dhaliwal, A.K. 2009. Recurrent phenotypic selection for increased winter productivity in annual ryegrass (Lolium multiflorum Lam.). Masters Thesis, Agronomy and Soils, Auburn University, Auburn, AL.


Table 2.1: Additive variance, phenotypic variance and narrow sense heritability values of dry matter yield in annual ryegrass for four harvest done at every 500 growing degree days (GDD) under harvest scheme 1 across three cycles of selection, in year 2009 at Plant Breeding Unit (PBU) in Tallasseo, AL and in year 2010 at two locations, PBU and the Alabama Agricultural Experiment Station Wiregrass Research and Extension Center at Headland (WGS), AL.

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80
Fig. 2.1: Narrow sense heritability values of dry matter yield in annual ryegrass for four harvests done at every 500 growing degree days (GDD) under harvest scheme 1 across three cycles of selection, in year 2009 at Plant Breeding Unit (PBU) in Tallassee, AL.
Table 2.2: Additive variance, phenotypic variance and narrow sense heritability values of dry matter yield in annual ryegrass for two harvest done at every 1000 growing degree days (GDD) under harvest scheme two and for cumulative yield of comparable harvests under harvest scheme one across three cycles of selection, in year 2009 at Plant Breeding Unit (PBU) in Tallassee, AL.

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Fig. 2.2: Narrow sense heritability estimates of dry matter yield in annual ryegrass for two harvests done at every 1000 growing degree days (GDD) under harvest scheme two, across three cycles of selection, in year 2009 at Plant Breeding Unit (PBU) in Tallassee, AL.
Table 2.3: Additive variance, phenotypic variance and narrow sense heritability values of dry matter yield in annual ryegrass for two harvests done at maturity under harvest scheme three across three cycles of selection, in year 2009 at Plant Breeding Unit (PBU) in Tallassee, Al and for one harvest in year 2010 at two locations, PBU and the Alabama Agricultural Experiment Station Wiregrass Research and Extension Center at Headland (WGS), AL.

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Fig. 2.3: Narrow sense heritability estimates of dry matter yield in annual ryegrass for one harvest at maturity under harvest scheme three, across three cycles of selection, in year 2010 at two locations, PBU and the Alabama Agricultural Experiment Station Wiregrass Research and Extension Center at Headland (WGS), AL.