Preliminary evaluation of selection criteria for drought resistance in alfalfa (Medicago sativa L.)
by John Robert Carlson Jr

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Philosphy in Crop and Soil Science
Montana State University

Abstract:
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Selection for seedling root pattern and for resistance to wilting indicated that these traits were lowly heritable. Selection for these traits will result in slow progress.

Selection for leaf color, leaflet size, and germination salt tolerance indicated that these traits were highly heritable. Selection for these traits will result in rapid progress.

PRELIMINARY EVALUATION OF SELECTION CRITERIA FOR DROUGHT RESISTANCE IN ALFALFA (*MEDICAGO SATIVA* L.)

by

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A thesis submitted in partial fulfillment
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ABSTRACT

Drought reduces alfalfa yield and quality. The objectives of this study were to: 1) develop screening procedures to assay alfalfa germplasm for drought resistance traits, and 2) determine if genetic progress could be made for these traits.

Traits studied included stomatal density, seedling root pattern, resistance to wilting, leaf color, leaflet size, and germination salt tolerance.

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CHAPTER I

INTRODUCTION

Alfalfa (Medicago sativa L.) is the most important forage crop in the United States and the world. It has been cultivated in the United States for nearly 250 years and has the highest feeding value of all commonly grown hay crops. Alfalfa possesses a broad range of adaptation which has enabled its successful establishment worldwide. Although alfalfa is tetraploid and highly self-incompatible, its breeding has been highly successful.

Moisture is one of the most important factors limiting alfalfa growth. Drought reduces yield and quality. It is complex and there is little information on the factors affecting drought resistance in alfalfa. There is also a lack of efficient selection techniques to screen large populations of alfalfa required to make genetic progress for drought resistance. To develop drought-resistant alfalfa cultivars selection criteria must be identified and incorporated into alfalfa breeding programs.

The objectives of this study were to: 1) develop efficient screening procedures for physiological and morphological traits related to drought resistance of alfalfa, and 2) determine if progress through divergent selection can be made for these traits.

CHAPTER II

LITERATURE REVIEW

Alfalfa (Medicago sativa L.) was the first domesticated forage crop (34). Alfalfa, now worldwide in distribution (38, 112), probably originated in the Near East and/or Central Asia (34). It is believed to have been first cultivated in Iran, and the first documented attempt to grow alfalfa in the United States was in Georgia in 1736 (112). It is now the most important forage crop in the United States and the world, and it has the highest feeding value of all commonly grown hay crops (112).

In the semi-arid western plains, moisture is one of the most important factors limiting plant growth and breeders have been trying to develop drought-hardy forage crop cultivars for dryland production (26, 36, 91, 195, 232, 303). Drought reduces crop yield, quality, plant height, and vigor (214), and influences almost all physiological processes (207). Although alfalfa is relatively drought resistant due to its extensive root system (49) and its ability to harden during periods of drought (43), there is little information on the factors affecting drought resistance (139).

Drought resistance is complex, involving interrelationships between plant and environmental factors (117, 132, 148, 209, 237). It presents a challenge to plant breeders as drought resistance must first be identified and then transferred to high-yielding and agronomically-acceptable cultivars (27, 51). Keim and Kronstad (145) and Parsons

(209) describe the ideal cultivar as possessing a relatively high yield in dry environments without sacrificing yield in environments where adequate moisture is available. Perhaps breeders should not strive for an "ideal" cultivar, but should develop cultivars adapted for specific dryland environments.

Although alfalfa is tetraploid and highly self-incompatible, its breeding has been highly successful (33, 34, 188). Progress has been made in improving alfalfa yields, quality, longevity, seed productivity, and resistance to diseases and insects (280). Progress in any breeding program is dependent on how accurately the breeder is able to identify and select superior plants from large populations and this is determined by the genetic variability in the population and the heritability of the desired trait(s) (12). Thus, any effort to improve drought resistance through genetic manipulation will not only require the proper selection criteria but also a reliable procedure to screen available germplasm (238). The selection procedure, which would most likely include a combination of traits (191, 209, 237), should be simple, rapid, and enable the efficient screening of large populations (78, 198).

The Influence of Drought on Plant Growth

Viets (284) defines drought as a "sustained period of significantly subnormal water or soil moisture supply." Low rainfall is the basic cause of drought, but other meteorological factors (e.g., wind,

air temperature, radiation, etc.) also contribute to water deficit (256). In dryland agriculture most of the water loss is due to evaporation either directly from the ground surface or indirectly via transpiration (evapotranspiration) from plants.

Most physiological processes in plants are affected by water deficits (155). Most of a plant's fresh weight is comprised of water (288). Kramer (155) states that water serves four general functions in plants. It is 1) a major constituent of physiologically active tissue, 2) a reagent in photosynthesis and hydrolytic processes (e.g., starch digestion), 3) the solvent in which sugars, salts and other solutes move in the plant, and 4) important in maintaining turgor for cell enlargement and growth.

Water stress has its greatest influence on rapidly growing tissues (302). Germination and rate of initial seedling growth are significantly reduced by moisture stress (103, 150, 170). Germinating seeds are particularly susceptible to water stress which may alter their metabolism to the point where internal processes delay or even halt germination (265). Root initiation may be delayed and subsequent root growth inhibited by water stress (110). Species differ considerably with respect to their drought tolerance during germination (245).

Water stress may retard or stunt plant growth (3, 11, 13, 38, 164, 214, 230, 248). Drought at different stages of development affects growth in different ways (69, 212). The organ growing most rapidly at the time of stress is the one most affected (16, 92). Reduced leaf

area (1, 38, 47, 166, 220, 273, 298), fluctuations in stem diameter (45), stomatal closure (10, 11, 31, 220, 227, 259, 268), increased senescence (136, 166, 171), and associated reductions in overall yield and vigor (3, 11, 13, 38, 71, 72, 89, 92, 99, 154, 166, 179, 242, 248) may occur in the presence of water deficits. Growth may be stopped with slight drops in tissue water potential since cell division and cell expansion are affected by even a prolonged mild stress (89, 90, 95, 101, 273). Structural changes in the protoplasm (294) due to water loss in cells, inducing mechanical stresses, are believed to be a major cause of drought injury (234).

Water stress reduces both yield and quality in alfalfa (71, 99). Low yields are due to less leaf tissue, reduced leaf area, and smaller, more densely packed leaf cells (38). Drought results in fewer stems per plant, fewer internodes per stem, reduced internode length, less regrowth, and impaired root growth in alfalfa (118, 214, 217).

Drought influences many of the physiological processes in plants. Reduced efficiency and rate of photosynthesis (4, 20, 45, 104, 166, 290, 304), increased respiration (39, 84, 155, 166, 250), reduced protein synthesis (64, 116, 242, 244), altered nitrate reductase activity (4, 203, 220), increased activity of peroxidase (275) and other enzymes (8, 58, 242), and reduced nutrient uptake (3, 92, 290, 305) are characteristic of water-stressed plants. Alexander et al. (8) determined

that measuring enzyme levels alone was not a sufficiently sensitive procedure to indicate water status when water supply is altered. Measurement of several physiological processes (e.g., photosynthesis, respiration, nitrate reductase activity) might prove a better index of plant water status (4).

Internal water balance is the most important aspect of plant water relations. Internal water balance and turgidity are affected by a complex combination of environmental factors (77, 139) and are related to the rates of several physiological processes (154), which are all adversely affected by water stress (208). Maximum crop yields are dependent on the uninterrupted maintenance of adequate moisture supply throughout the growing season (256); thus, magnitude, time, and duration of water stress become important in determining how well the plant recovers (3, 54).

Blum (29) believes that present physiological knowledge of plant responses to water stress is sufficient for the initiation of breeding programs for resistance to water deficits. In order to capitalize on this knowledge, breeders must determine which physiological criteria are most important in conditioning drought resistance and how efficiently they may be implemented into breeding programs to insure significant and rapid progress.

Resistance to Drought and Its Measurement

General Forms of Drought Resistance

Many comprehensive reviews summarize the drought and drought resistance literature (17, 29, 93, 115, 119, 124, 125, 126, 135, 138, 154, 155, 164, 167, 168, 169, 180, 189, 206, 209, 234, 246, 261, 262, 276, 278, 283, 284, 302). However, there has been very limited success in breeding for drought resistance (139, 182). Genetic variability has been observed for drought resistance within almost all agronomically-important crops. Some plants can even survive after almost complete tissue dehydration without serious damage (88).

Russell (234) defined drought resistance as "the overall suitability of plants for cultivation under dry conditions." Wilson et al. (288, 299) define drought resistance as a plant obtaining "the maximum supply of water from the roots compared with that needed for survival." There are three general categories of drought resistance in plants (11, 169, 261, 264). Drought escape involves completion of the plant's life cycle during a short, favorable period when sufficient moisture is available. Drought avoidance is the ability of the plant to evade drought injury by leaf shedding, stomatal closure, development of a thick cuticle, reduced growth, or any other mechanism which "shields" the plant from desiccation. Drought tolerance (261) or hardiness (48, 234) is the ability of a plant to survive varying degrees of tissue

desiccation. All three of these mechanisms may be present to some extent in a plant (261), but avoidance is the most common type employed by agronomically-important crops (27, 28, 226, 236, 259, 263, 298, 299) so that photosynthesis and growth may proceed despite environmental water stress.

Drought resistance may be related to winterhardiness and salt tolerance since all three conditions may involve similar protoplasmic effects (165, 167, 169, 173). Resistance to one type of stress may imply resistance to the other (167). Unfortunately, there may be a negative correlation between drought resistance and yield in many instances (29) even though a plant which does not survive obviously does not yield. Smaller, low-growing plants are less affected by drying winds and have less evaporative surface area, thus avoiding desiccation. Slow regrowth after cutting and bud dormancy are contributing factors to dryland survival in alfalfa (57, 151) and several forage grass species (18, 179, 190) which may result in lower yields when moisture is available.

Morphological and Anatomical Drought Resistance Factors

Among the initial studies dealing with the problem of drought resistance in plants were those pertaining to morphological characteristics. Morphological characteristics which reduce water loss help plants avoid desiccation and could be selected to develop drought-resistant alfalfas.

1. Topgrowth Factors

Plants with thick cuticles (11, 154, 213, 253), increased pubescence (98, 300), and decreased leaf area (11, 231, 267, 268, 298) have been purported to be drought resistant. Thick, waxy cuticles resist moisture loss through epidermal cells not associated with stomata, and pubescence slows air movement and reduces transpiration. Reduced leaf area results in a smaller evaporative surface, thus conserving water. Plants with succulent leaves avoid desiccation by maintaining a high moisture content during drought (167, 213).

Leaf color may also be important in conditioning drought resistance. Dark leaves used water more efficiently than pale leaves in studies with barley (Hordeum vulgare L.) (2). Pale-leaved lines possessed lower canopy temperatures, higher albedos, higher net radiation, and greater sensible heat loss but yielded significantly less than dark-leaved lines (2, 81, 82). The cooler canopies in pale-leaved barley lines are probably due to increased reflection (81). Variability exists for this trait in alfalfa, so this trait could be implemented into a breeding program for drought resistance.

There are many studies (4, 10, 32, 41, 47, 50, 53, 59, 61, 67, 85, 87, 113, 121, 127, 128, 134, 137, 141, 166, 167, 183, 186, 191, 194, 201, 213, 219, 227, 240, 241, 247, 259, 266, 268, 269) on stomata and their relationship to drought resistance. Plants with lower stomatal densities are generally thought to be more drought resistant (67, 134).

Jones (134) suggests selection for smaller pore size and lower densities to improve drought resistance in barley. These traits are heritable in some species (32, 113, 186), but other studies (128, 191) have failed to reveal a correlation between density and/or size and transpiration.

Alfalfa leaves developed in the sun have more stomata per unit area than those developed in shade (53), and adaxial leaf surfaces have more stomata than do abaxial surfaces (50, 53). Cole and Dobrenz (50) compared stomatal densities of seven alfalfa cultivars by sampling five microscopic fields from the abaxial and adaxial surfaces of single leaflets from the top four nodes on one stem from each of two plants per cultivar in four replications. Significant differences as small as 9% were detected among cultivars for stomatal densities on the adaxial surfaces. Larger differences among cultivars were needed to detect significant differences for the abaxial surfaces. The importance of within-plant variability influencing stomatal density in alfalfa has not been determined.

Stomatal behavior is probably more important in enabling plants to avoid desiccation than either density or pore size. The ability of a plant to swiftly close its stomata during brief dry periods is a measure to conserve water even though growth may be curtailed for a short time (4, 87, 127, 166, 194, 220, 259, 268). Leaf diffusion porometers measure resistance to gas flow (4, 28, 76, 108, 249), and are used to monitor stomatal behavior. Stout and Simpson (259)

suggested that stomatal closure is only important when water stress becomes severe. Some of the drought resistance of alfalfa may be accounted for by its characteristic "midday closure" (183) of stomata, but Muenscher (191) concluded that the differential water loss observed among species is governed by a complex of factors and not just the stomata.

2. Root Factors

A large and well-developed root system is conducive to plant survival in dry areas (7, 40, 42, 62, 66, 111, 123, 124, 149, 167, 216, 221, 235, 268, 272). Extensive roots make it possible for plants to use available moisture from a large root zone (222, 272). Roots may be studied dynamically by using radioactive dyes such as P^{32} (105) or with glass-faced growth boxes (192).

Cohen and Strickling (49) found that the most important moisture for crop use in alfalfa was that in soil horizons close to the surface, so deep-rootedness might not necessarily be an advantage under certain conditions. Dittmer and Tally (65) suggest that plants possessing an extensive shallow root system supplemented by a deep, vertical one possess the greatest water use efficiency. Alfalfa has been observed to deplete moisture uniformly at several depths (79). Persistent root hairs and the ability of a plant to secrete mucilaginous substances have also been implicated in drought resistance (216). Smaller, less

extensive root systems have even been purported to be better, especially during long drought periods (156, 225).

Higher root/shoot ratios are considered to be advantageous for dryland survival (197, 200, 211, 226). Slight decreases in soil water potential may significantly increase root/shoot ratios (226). Root/shoot ratios, however, may be greatly influenced by several environmental factors (211) such as moisture, temperature, and soil type, so the heritability of this trait could be low.

Although considerable variability exists for rooting habit in alfalfa (243, 254), observation of root growth is difficult, tedious, and little is still known about what constitutes an effective root system (228). Drought resistance is most likely not simply related to differences in rooting depth or other rooting characteristics as has been suggested by Steckel and Gray (257) for potatoes (Solanum tuberosum L.).

3. Anatomical Factors

Anatomical characteristics in plants have also been studied (28, 38, 56, 80, 100, 152, 167, 199, 234). Drought-tolerant plants have been purported to have smaller, more densely-packed cells (38, 56, 65, 167, 234) since smaller cells, when desiccated, undergo a smaller proportionate reduction in volume (234). Cell wall elasticity may also be important in preventing desiccation injury (28). Modified vascular anatomy in the roots such as increased wall thickening in the endodermal

cells, well-developed root xylem, wider Casparian strips, and a reduced cortex (80, 100) would most likely increase the efficiency of water transport (199). A well-developed vascular system allows for better translocation of photosynthates and water (38).

Morphological and/or anatomical characters are hard to directly relate to drought resistance or yield (32). Since drought resistance is governed by a complex of characters (237, 234), it would probably not be advantageous simply to rigidly select for one or more morphological and/or anatomical traits to isolate superior germplasm (68) but to use these traits in conjunction with drought resistance screening and evaluation techniques. Once heritable, adaptive morphological plant characters are identified, they can be implemented into breeding programs (81).

Physiological Drought Resistance Factors and Selection Techniques

Elaborate procedures to measure drought resistance in crop plants have been described (15, 27, 28, 35, 76, 146, 156, 157, 159, 181, 286). Although these procedures are useful in identifying drought-resistant plants, simpler techniques are needed to more rapidly assay large, segregating populations (35, 78, 198). Seedling screening techniques that correlate to mature plant resistance would be very valuable since a minimum of time and space would be involved (237).

1. Germination Under Stress

Several attempts have been made to relate germination in sodium chloride, mannitol (163), polyethylene glycol, and similar solutes to drought resistance or winterhardiness. The most common selection technique has been to place seed on blotters or filter paper in closed containers and to moisten with salt solution (44, 73, 282, 305). Others (14, 174) have used saline soil or water cultures to measure salt tolerance of germinating seeds. Workman and West (301) used agar containing NaCl to test germination in winterfat (Eurotia lanata (Pursch.) Moq.). They observed genetic differences within the species which enabled certain strains to better germinate at higher NaCl levels.

Croughan et al. (55) studied cell growth of alfalfa grown in agar containing 1% (w/v) NaCl. They observed differences in growth between a salt-tolerant line of alfalfa and a nonselected line from the same original population. They suggested increased NaCl tolerance in the selected line due to its better relative growth at high levels of NaCl. Since the salt-tolerant line performed poorly in the absence of NaCl, they hypothesized that a substantial amount of NaCl was required for optimal growth.

Plants are usually most sensitive to osmotic stress during germination and early seedling development (14). Osmotic stress, induced by saline conditions, affects plant growth similarly to drought stress such as increasing root/shoot ratios (44), decreasing yields (14, 74,

86, 109, 130, 160, 196, 208, 288), and delaying or reducing germination (14, 52, 70, 73, 114, 150, 174, 175, 245, 260, 282, 295, 297). Although genetic variability exists for salt tolerance (24, 83, 144, 245, 298), the relationship between salt tolerance and drought tolerance is controversial (178). No correlation has been found between ability to germinate in osmotic solutions and drought tolerance in mature plants (172, 245, 305) probably because osmotic substrates induce effects, including toxicity, more complex than simple drought (130, 184, 282). Thus, even though alfalfa is relatively salt tolerant (37, 153), the same mechanisms may not condition drought tolerance.

2. Water Potential Measurements

Water retention of plant tissues is one of the simplest measurements used in assessing a plant's ability to avoid desiccation (158). Hydrophillic colloids in the plant cytoplasm are purported to be the most significant factor in water retention (195). Dedio (60) observed genotypic differences in wheat (Triticum aestivum L.) for water retention in excised, drying leaves. Martin (179) found that stalks of sorghum (Sorghum bicolor L.), a relatively drought-resistant crop, dried more slowly than those of corn. Teoh (270) suggests that drought-tolerant plants lose water less readily than susceptible plants when they are cut. However, Salim and Stutte (236) and Sandhu and Laude (239) suggest that water retention may provide only a "fair" means of assessing drought tolerance.

Leaf water potential measurements are more complex and time-consuming. Instruments such as pressure bombs (35, 63, 291), psychrometers (35, 152, 176, 177, 187, 224), and beta gauges (181, 193) measure water potential. Many studies (6, 27, 28, 31 35, 76, 143, 148, 152, 210, 292, 293) relate water potential and related factors (matric potential, turgor pressure, suction tension, and relative water content) to plant water status and drought resistance in plants. Blum (27) measured leaf water potential in sorghum periodically during the growing season and found genotypic differences in drought resistance. Al-Saadi and Wiebe (6) determined that different species had different capacities to bind water by matric forces, probably due to different properties of colloidal materials. However, the correlation between drought tolerance and matric potential was low and variable. Water potential measurements provide useful and accurate estimates of plant water status, but are too time-consuming to use for the screening of large, heterogeneous plant populations.

3. Concentration of Ethylene and Abscissic Acid

Increased concentrations of ethylene and abscissic acid initiate leaf abscission and may be an index of drought resistance. The role of ethylene and abscissic acid has been studied in many species (5, 21, 23, 25, 75, 102, 162, 215, 218, 223, 229, 289) and their accumulation often results from water stress (22, 136, 171, 229, 289). Ben-Yehoshua and

Alohi (22) found that as water stress increased, more ethylene was produced in 'Valencia' oranges (Citrus simensis Osbeck.). McMichael et al. (171) evaluated the effects of water deficits in cotton (Gossypium hirsutum L.) and found a linear relationship between ethylene production, leaf abscission, and water deficit. Water stress was also related to a decrease in auxins and enhanced production of hydrolytic enzymes in the abscission zone. Walton et al. (289) discovered an association between water stress and abscissic acid levels in bean roots (Phaseolus vulgaris L.).

Both ethylene and abscissic acid can be readily measured. Assessment of their concentration following drought stress may be useful in a breeding program for drought resistance.

4. Proline Accumulation

Free proline accumulates in the leaves of many species following water stress (19, 30, 106, 107, 118, 120, 204, 205, 233, 251, 277, 279, 285, 286). Large proline accumulations following water stress may be a positive index of drought tolerance (19, 30, 261, 285). However, Waldren et al. (286) point out that proline accumulation is not a very sensitive indicator of drought stress, since it only accumulated after yield was severely affected in sorghum and soybeans (Glycine max Merr.). Tymms and Goff (279) found that proline accumulation was not related to the extreme drought tolerance of "resurrection" plants. Hanson et al.

(106, 107) suggest that proline-accumulating potential should not be used as a positive index of drought tolerance as the massive proline accumulation in barley is merely a symptom of severe water stress and has no survival value during drought. They further suggest that selection for high proline accumulation may even result in a shift in the population in the direction of drought susceptibility. Even though proline-accumulating ability may be a heritable trait, isolation procedures currently in use are too time-consuming for use in initial germplasm screening. Measurement of proline accumulation may be useful for evaluation of prospective drought-tolerant cultivars selected by other methods.

5. Other Techniques

Recovery following drought stress, whether artificially imposed or in the field, has been used to assess drought tolerance (13, 42, 54, 116, 122, 156, 167, 185, 196, 198, 202, 274, 298). In 1936, Hunter et al. (122) simulated drought under laboratory conditions by withholding water. They were able to distinguish among eight corn (Zea mays L.) inbred lines differing in drought resistance and field data confirmed the validity of their procedure. Todd and Webster (274) simulated repeated drought periods in the greenhouse and results correlated well with known field drought resistance for several cereals.

O'Toole et al. (202) withheld water for 10 days from rice (Oryza sativa L.) seedlings in a growth chamber and found differences in survival among plants. Nour et al. (198) subjected sorghum seedlings to four successive cycles of water stress in a growth chamber and measured percent survival. They concluded their technique to be a simple and effective screening method. However, Chinoy (46) points out that severe stress may not be useful in showing cultivar differences.

Osmotic substrates, such as polyethylene glycol (108, 129, 131, 142, 144, 161, 207, 208, 271) are used to simulate drought in the laboratory or greenhouse. Johnson and Asay (131) tested emergence of 120 crested wheatgrass (Agropyron spp.) lines under polyethylene glycol-induced drought stress. Cellulose acetate membranes were used to avoid direct contact between growth media and osmoticum. They found differences among lines for emergence under the imposed stress. However, selection under artificially-imposed stress in the greenhouse resulted in low correlations with field drought resistance (133) since field conditions were more complex and variable (137).

Kaloyereas (140) proposed the chlorophyll stability index (CSI) as a possible measure of drought resistance of pine (Pinus taeda L.) seedlings. The less readily chlorophyll degraded under heat stress, which often accompanies drought (94), the higher the drought resistance.

Kilen and Andrew (147) measured CSI to assess drought tolerance in sweet corn (Zea mays L.). Susceptible lines had an index of 5.6, while resistant lines had an index of 1.6, in a test involving 12 dent and sweet corn lines differing in phenotypic response to field drought. Chlorophyll concentration can be easily measured with portable reflectance meters (287) so this characteristic may warrant further consideration as a screening tool.

Genkel et al. (96) and Genkel and Schelamova (97) believe that starch hydrolysis in the roots of many species is negatively correlated with drought resistance. Excised root tips were stained with Lugol's solution following desiccation and were then rated on a 1-4 scale under the microscope for starch hydrolysis. Seedlings in which less than 30% starch hydrolysis took place following an artificial desiccation stress were considered drought tolerant. The authors suggest that this method is reliable for selecting drought-tolerant cultivars.

Kessler (46) described the relationships between cell size, nucleic acid fraction and the tolerance of bean and pea plants to heat and water stress. He concluded that the plants with greater heat and drought tolerance had more adenine. The DNA to RNA ratio also increased with greater drought tolerance.

The problem of drought has presented a challenge to plant breeders in obtaining yield improvement in drought-stricken regions without

irrigation (27, 124). Drought resistance is very complex and little progress has been made through breeding. In order to be successful in developing a dryland alfalfa, heritable characteristics conditioning drought resistance must first be identified and later incorporated into high-yielding and agronomically acceptable cultivars.

CHAPTER III

SAMPLING STOMATAL DENSITY IN ALFALFA

The relative importance of within-plant variability influencing stomatal density in alfalfa has not been determined and sample size requirements are not documented. The objectives of this study were to determine 1) the magnitude of within-plant variability for stomatal density associated with stems, leaf position, and microscopic fields in alfalfa; 2) optimum allocation of sampling resources to obtain maximum precision for detecting differences among individual genotypes; and 3) whether within-plant variability was too large to use stomatal density as a possible rapid screening criterion in breeding for drought resistance in alfalfa.

Materials and Methods

Five alfalfa plants were randomly chosen in 1978 from a 1977, Bozeman, Montana planting of diverse progeny rows. The plants had been harvested once and were at the early bud stage. Leaf samples were taken from 10 stems per plant from three successive nodes. Node 1 was the first node from the top of the plant that appeared to have fully expanded leaves. Nodes 2 and 3 were immediately below node 1. The two lateral leaflets from each trifoliolate leaf were removed and the adaxial surface of one and the abaxial surface of the other were sprayed with acrylic Tuffilm. The spray was allowed to dry for

5 minutes before applying clear plastic tape to the sprayed leaf surface. The hardened Tuffilm was peeled from the leaflet and taped to a microscope slide.

A microprojector was used to count stomata from 10 random microscopic fields (0.128 mm^2 per field), 5 on each side of the midvein. If at least one-half of a stomate was in view, it was counted as in the field. Fields obscured with vascular tissue, epidermal tears or other obstructions were not sampled. Epidermal cells were counted simultaneously from a 0.03 mm^2 pie-shaped section from the same fields used for the stomatal counts. Portions of epidermal cells within the field were rounded to the nearest one-fourth and were added to the number of whole cells present in the field to arrive at total epidermal cells per 0.03 mm^2 field.

Analyses of variance were conducted for stomatal density at each node and then combined over nodes. Stems and fields were considered as random and plants and nodes as fixed effects in the analysis. Variance component estimates for within-plant sources of variation, stems, and fields, were obtained by equating observed mean squares to their expectations and solving for the appropriate variance components.

Various sampling combinations were compared to the specific case of sampling 10 fields per leaflet, 3 nodes per stem, and 10 stems per plant. Relative efficiency was defined to be (255):

$$\hat{\sigma}^2_{\bar{y}} / \left(\frac{\hat{\sigma}^2 S/P/N}{rn} + \frac{\hat{\sigma}^2 F/S/P/N}{rnm}\right)$$

where: $\hat{\sigma}^2_{\bar{y}}$ = variance of a plant mean with 10 stems per plant and 10 fields per node on each of 3 nodes;

$\hat{\sigma}^2 S/P/N$ = variance component for stems from the combined analysis of variance;

$\hat{\sigma}^2 F/S/P/N$ = variance component for fields from the combined analysis of variance;

m = number of fields per node;

n = number of stems per plant; and

r = number of nodes per stem.

The within-plant variance components were also used to estimate sampling requirements needed to detect pre-chosen differences between plants fulfilling given probability levels for Type I (α) and Type II (β) errors using the formula

$$n = 2(z_\alpha + z_\beta)^2 S^2 / d^2$$

where: n = number of stems;

z_α = the z value corresponding to the two-tailed significance level α for a normal variable; and

z_β = the t value corresponding to the desired probability $1-\beta$ of detecting a significant result if the true difference, expressed as a percent of the mean, is d.

Results and Discussion

Significant differences were observed among plants for stomatal density at each node and combined over nodes on both the abaxial and adaxial leaflet surfaces (Table III-1). Stomatal density decreased from node 1 to node 3. Although data are not presented, epidermal cells were counted along with stomata. The ratio of stomata to epidermal cells increased from upper to lower nodes (0.225, 0.239, and 0.250 for abaxial leaflet surfaces and 0.242, 0.254, and 0.267 for adaxial leaflet surfaces at nodes 1, 2, and 3, respectively). Plant x node interaction was not significant for either leaf surface indicating that relative differences among plants are similar provided that leaflets from the same nodes are sampled on all genotypes.

The two within-plant variance components were always less for the abaxial leaflet surface at any given node (Table III-2). In general, within-plant variability declined from node 1 to node 3.

Because efficiency of different sampling schemes was nearly identical for both leaflet surfaces, only data for the abaxial surface are plotted in Figure III-1. An efficiency of 100 percent has been arbitrarily defined to be the sampling of 10 fields on leaflets from 3 nodes on each of 10 stems. Increasing the number of fields sampled from leaflets on a given stem has little effect on increasing efficiency compared to sampling leaflets from more stems. In practice one should

Table III-1. Mean stomatal densities (stomata per mm^2) of five alfalfa plants at three successive nodes.

Plant	Node 1		Node 2		Node 3		Mean	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
1	196.4	240.3	192.0	236.9	177.4	217.3	188.6	231.5
2	155.1	192.3	157.3	186.0	147.1	175.3	153.2	184.5
3	167.6	234.6	166.6	225.7	161.5	217.7	165.2	226.0
4	180.6	221.3	173.5	215.3	167.1	204.4	173.7	213.8
5	206.8	238.3	191.9	234.3	181.1	219.5	192.3	230.7
LSD (0.05)	16.3	22.0	16.1	18.2	14.8	17.5	13.0	15.8
Mean	181.3	225.4	176.3	219.6	166.8	206.8	174.6	217.3

Table III-2. Within-plant components of variance for alfalfa stomatal density (stomata per mm^2) for three successive nodes.

Node	Stems $\hat{\sigma}_S^2$		Microscopic fields $\hat{\sigma}_F^2$	
	Abaxial	Adaxial	Abaxial	Adaxial
1	318±74	593±131	276±18	365±24
2	316±70	394± 90	212±14	375±25
3	261±59	372± 83	238±16	274±18
Combined	298±39	453± 59	242± 9	338±13

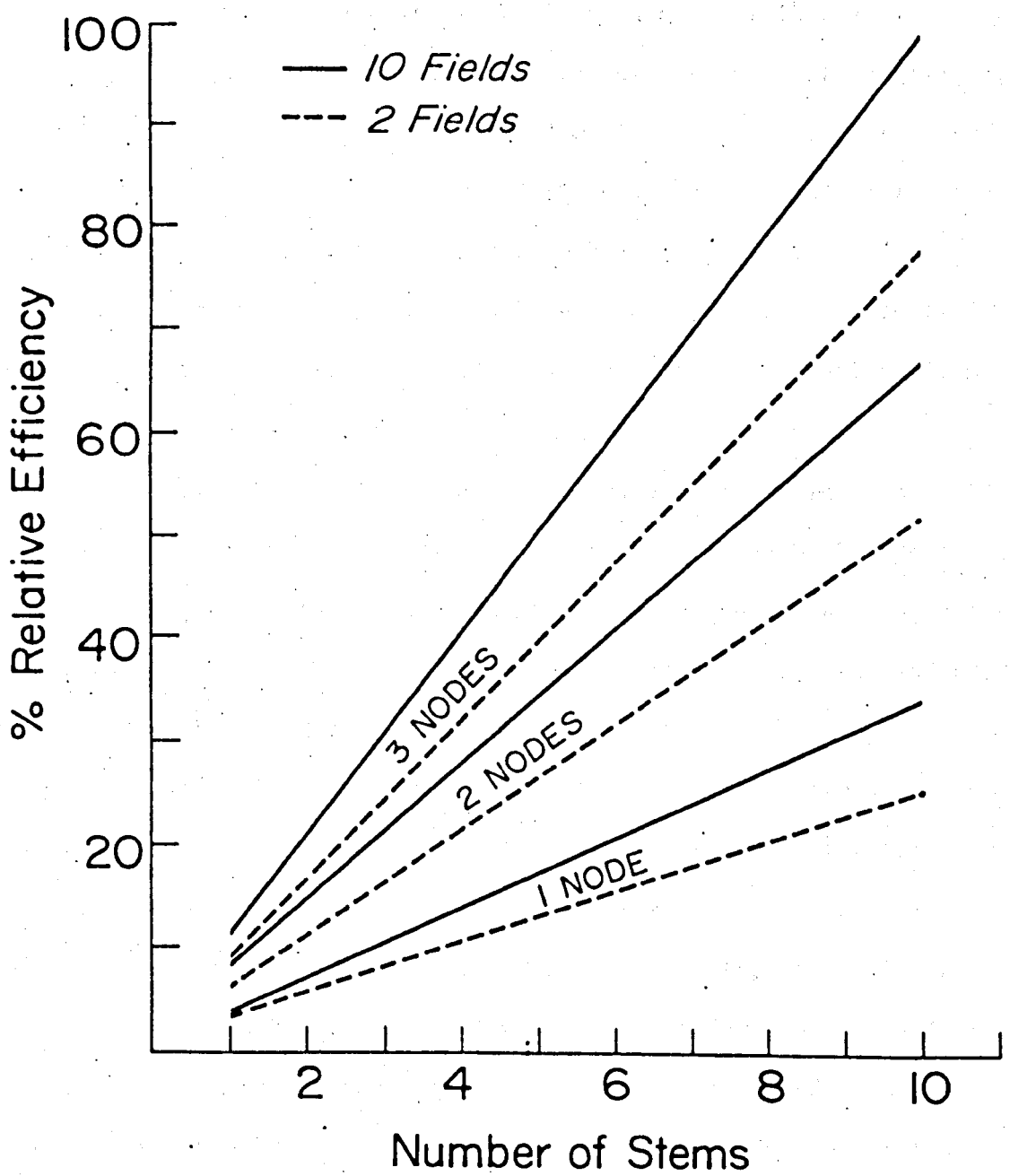

Figure III-1. Relative efficiencies for sampling stomata in alfalfa for one, two, and three nodes when 2 and 10 fields are counted on leaflets from 1 to 10 stems.

allocate samples to leaflets from more stems as opposed to more nodes on the same stem to insure that all leaflets are of similar age.

Sampling requirements needed to detect differences between plants, holding Type I and II errors at 0.05 and 0.10, respectively, are presented in Table III-3. Because node 3 is less variable and more mature, only stem and field combinations for node 3 are shown. These data again corroborate that sampling resources should be allocated to leaflets from additional stems as opposed to more fields on leaflets from the same stem. Sample sizes could be reduced if one is willing to accept larger Type I and Type II errors. However, for selection purposes the plant breeder is concerned with minimizing both Type I and Type II errors, since it is important to detect differences among genotypes when they do exist and to have those differences be real and not due to random chance.

Genetic variability exists for stomatal density; however, the considerable amount of within-plant variability necessitates the sampling of leaflets from an extremely large number of stems to discriminate among plants differing by less than 10%. As a result, stomatal density was not used as a selection criterion in breeding for drought resistance in alfalfa. Many factors affect stomatal density and this study was conducted at only one location and in one year. However, testing plants in more environments would probably further increase the number of total samples required.

Table III-3. Estimates of stem numbers required to detect a given difference (d) between any two alfalfa plants at node 3 for stomatal density when 4, 10, and 40 fields per leaflet are sampled on each side. 1/

d	Abaxial			Adaxial		
	4	10	40	4	10	40
%						
5	97	87	81	87	79	75
10	25	22	21	22	20	19
20	7	6	6	6	5	5
40	2	2	2	2	2	2

1/ $\alpha = .05$, $1-\beta = .90$.

CHAPTER IV

DIVERGENT SELECTION FOR SEEDLING ROOT PATTERN IN ALFALFA

Variability exists for rooting habit in alfalfa (243, 254), but little is known about what constitutes an effective root system (228). Some alfalfa seedling roots have many secondary roots and appear fibrous in nature. Others possess a single, vertical tap root with little or no branching. Since rooting habit is believed to be related to drought resistance, this trait could be used as a rapid selection criterion for alfalfa. The objectives of this study were 1) to determient the extent of the variability existing for seedling root pattern in alfalfa; 2) to determine if progress from divergent selection can be made; and 3) to estimate heritability of seedling root score.

Materials and Methods

Initial Selection

Ladak 65 alfalfa seeds remaining on a 0.13 cm round screen were planted 1 cm deep in 50 x 30 x 8 cm aluminum flats containing no 3 terra-lite. Each flat had 10 rows, 30 cm long, with approximately 25 seeds per row. The flats were watered daily with nutrient solution (2.3 g N, 2.3 g P_2O_5 2.3 g K_2O, and 18.9 l H_2O). Upon emergence the plants were thinned to 13 plants per row (130 plants per flat). At the unifoliolate leaf stage (10 days) the plants were carefully removed from the terra-lite and scored for rooting pattern using a 1-5 scale (1 = tap root

only, no secondary roots; 5 = tap root and numerous secondary roots) (Fig. IV-1). Only plants at the unifoliate leaf stage (1,409 plants) were scored to insure uniformity. Plant scoring "1" as well as "4's" and "5's" were saved to comprise cycle one "tap-rooted" (TR1) and cycle one "fibrous-rooted" (FR1) population, respectively. Selected plants were transplanted into 20-cm pots containing Bozeman silt loam soil and grown in the greenhouse at 22 C with a 16-hour photoperiod. Seed was increased in an isolated greenhouse room containing a hive of honey bees (beeroom). Each population was separately intercrossed and seed from each plant was individually harvested, cleaned, and packeted. Several successive isolated seed increases were needed to obtain enough seed for a cycle one progeny test for each population.

Cycle One Progeny Test

Seed from 38 TR1 and 38 FR1 plants were mechanically scarified for 5 seconds using a Forsberg scarifier containing sandpaper. The seed was planted 1 cm deep in replicated progeny rows in 50 x 30 x 8 cm aluminum flats containing no. 3 terralite. Six blocks of TR1 progenies and five blocks of FR1 progenies were seeded in separate experiments along with unselected Ladak 65 in a randomized complete-block design. Ten 30 cm rows were seeded in each flat with 25 seeds per row. The plants were grown in the greenhouse at 22 C with a 16-hour photoperiod and watered daily with nutrient water. Upon emergence, the plants were

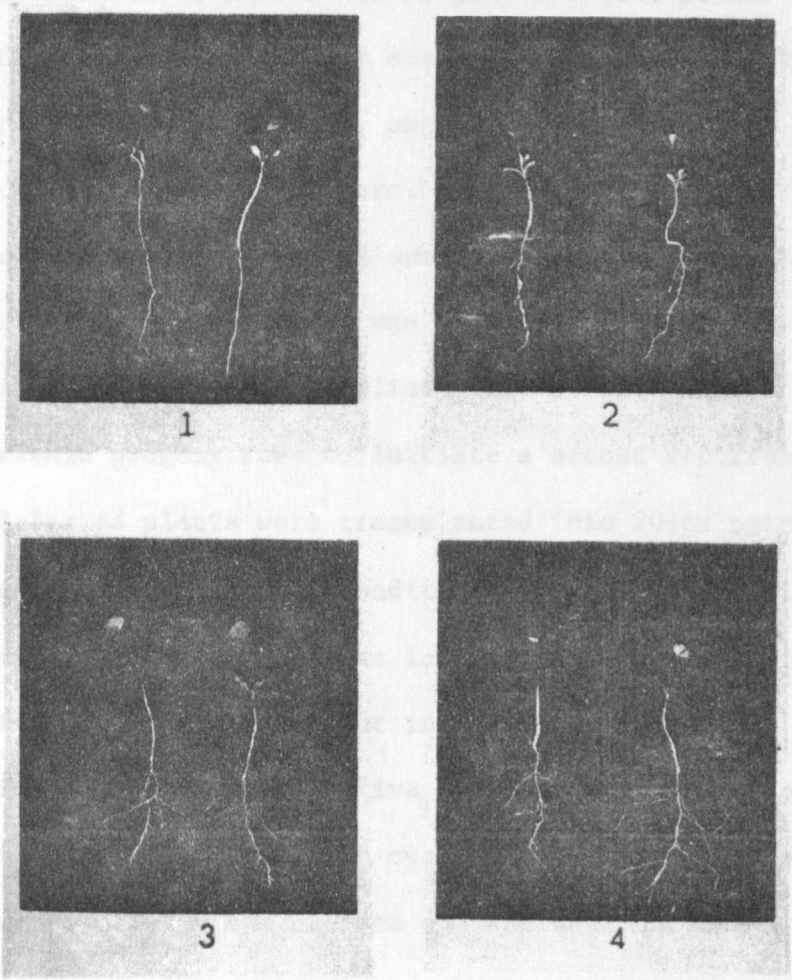

Figure IV-1. Illustration of the 1-5 scoring system (1 = tap root only, no secondary roots; 2,3,4 = intermediate types; and 5 = tap root and numerous secondary roots) used in evaluating alfalfa seedling roots at the unifoliate leaf stage.

thinned to 15 plants per progeny row (150 plants per flat). At the first trifoliolate leaf stage (14 days) the plants were carefully removed from the flats, one block at a time, and individually scored for seedling root pattern. A 1-7 scale (1 = tap root only, no secondary roots; 7 = tap root and numerous secondary roots) (Fig. IV-2) was used since more variability was observed at the first trifoliolate leaf stage than at the unifoliate leaf stage. Plants were selected from within progeny rows to initiate a second recurrent selection cycle. Selected plants were transplanted into 20-cm pots and grown in the greenhouse under the conditions previously described. At maturity each cycle two population was intercrossed separately in the beeroom and the seed from each plant individually harvested, cleaned, and packeted. Additionally, the top five surviving maternal clones (based on progeny test results) from each cycle one population were intercrossed separately in the beeroom and the seed harvested from individual plants.

Cycle Two Progeny Test

Seed harvested from 55 cycle two tap-rooted (TR2) plants, 49 cycle two fibrous-rooted (FR2) plants, several TR1 and FR1 progenies, unselected Ladak 65, and the five genotypically selected TR1 and FR1 plants (hereafter designated TRG1 and FRG1, respectively) were seeded in a replicated progeny test in the greenhouse using the previously described procedures. Twelve rows, 30 cm long, were planted per flat

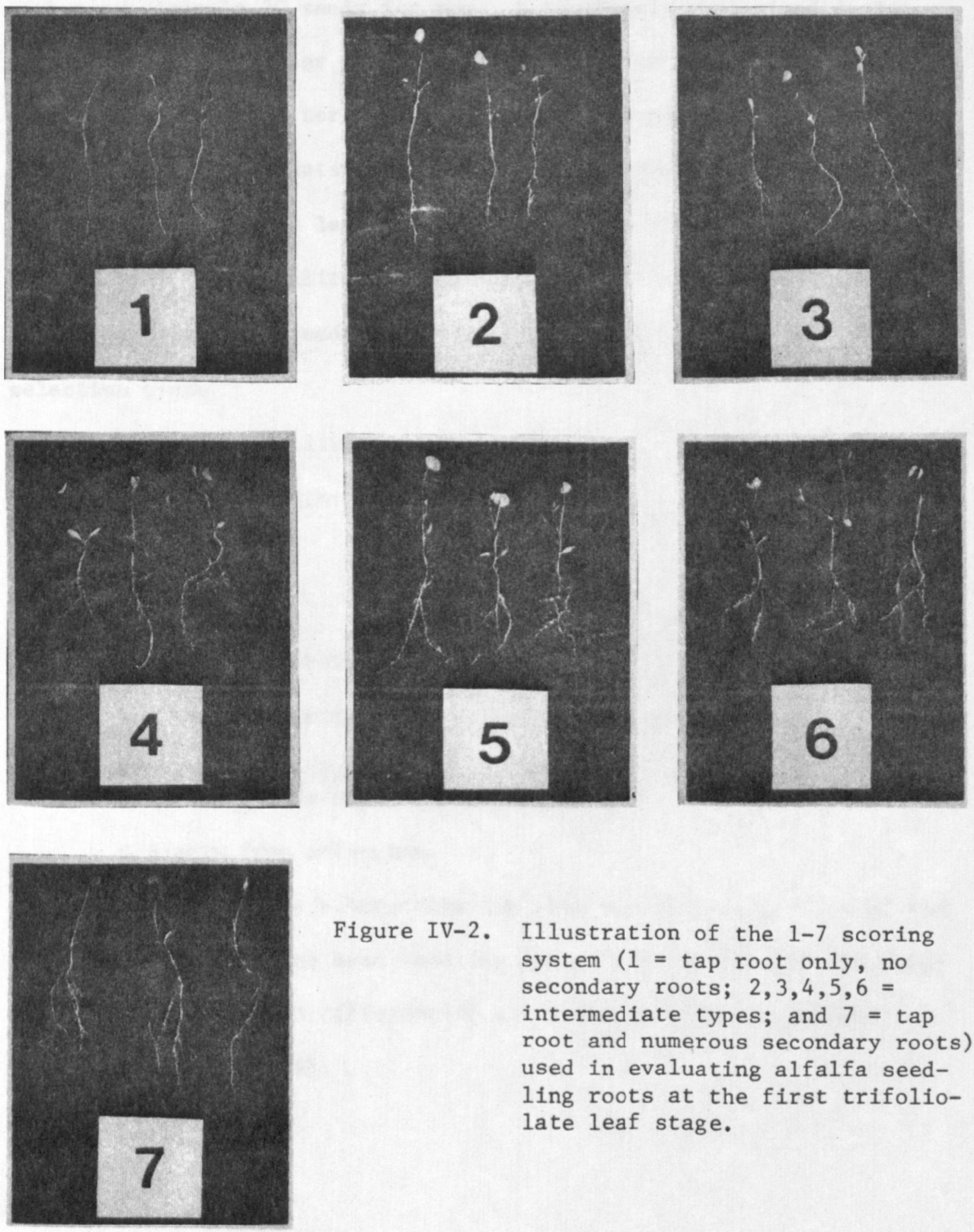

Figure IV-2. Illustration of the 1-7 scoring system (1 = tap root only, no secondary roots; 2,3,4,5,6 = intermediate types; and 7 = tap root and numerous secondary roots) used in evaluating alfalfa seedling roots at the first trifoliolate leaf stage.

with approximately 20 seeds per row. A completely-randomized design with four replicates per progeny was used and the plants were watered daily with nutrient water. Upon emergence, the plants were thinned to a maximum of 10 plants per progeny row (120 plants per flat). At the first trifoliolate leaf stage (14 days) the plants were carefully removed from the terralite and scored using the 1-7 scale (Fig. IV-2). Selections were again made within progeny rows to initiate the third selection cycle.

Realized heritabilities were calculated using a modification of the genetic gain formula (9):

$$H = G/(\sigma_A)(k) \qquad (1)$$

where: H = the heritability coefficient;

k = the standardized selection differential;

σ_A = the phenotypic standard deviation of the population the plants were selected from; and

G = gain from selection.

Gain was calculated by subtracting the mean seedling root score of the previous cycle from the mean seedling root score of the selected population. The selection differential was 2.06 based on a 5 percent selection intensity (9).

Cultivar and Germplasm Survey

Variability in seedling root scores was determined for 16 germplasms and 10 commercial cultivars (Appendix table 1). Seeds from each source were planted 1 cm deep in a greenhouse bench containing no. 3 terralite. A randomized complete-block design with four replications was used. Fifty seeds from each source were seeded in each block. The plants were watered daily with nutrient water and grown with a 16-hour photoperiod at 22 C. Upon emergence, the plants were thinned to 25 plants per row. At the first trifoliolate leaf stage (14 days) the plants were carefully removed from the terralite and scored using the 1-7 scale (Fig. IV-2).

Results and Discussion

Initial Selection

Seedling root scores were obtained from 1,409 Ladak 65 alfalfa plants. The mean seedling root score for all plants was 2.67 and flat means varied from 2.56 to 2.77. The distribution of seedling root scores was skewed toward the lower (tap-rooted) end of the curve (Fig. IV-3). There were six times as many "1's" as "5's." Selection intensity at each end of the distribution was 5 percent (71 "1's" and 68 "4's" and "5's" saved).

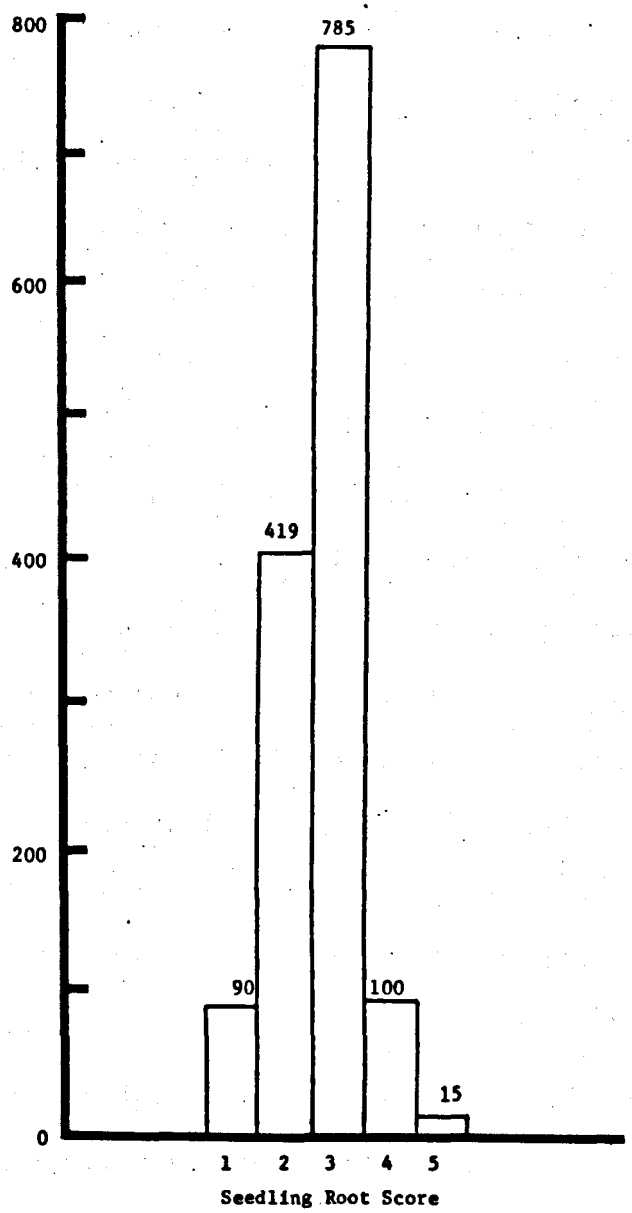

Figure IV-3. Frequency histogram for the initial screening of Ladak 65 alfalfa seedlings using a 1-5 seedling root score (1 = tap root only, no secondary roots; 5 = tap root and numerous secondary roots).

Cycle One Progeny Test

Two separate progeny tests, one involving 38 TR1 progenies and the other 38 FR1 progenies, were conducted. Unselected Ladak 65 was included as a check in both tests.

The mean seedling root score of progenies from the 38 TR1 clones was 3.30, significantly lower than the mean (3.71) of the unselected Ladak 65 plants (Table IV-1). Seventeen TR1 progenies had significantly lower seedling root scores than Ladak 65. The realized heritability of the tap-rooted habit was 11.4 percent. This low heritability indicates that environment plays a large role in the expression of this trait, and that progress through selection will probably be slow.

The mean seedling root score of progenies from the 38 FR1 clones was 3.98 and not significantly different than the mean (3.80) of the unselected Ladak 65 plants (Table IV-2). Four FR1 progenies had significantly higher seedling root score than Ladak 65. The realized heritability of fibrous-rootedness was 8.6 percent.

Significant block effects were detected for both TR1 and FR1 progenies. Plants evaluated later in the 2 to 3 day scoring period had higher seedling root scores than those initially evaluated (Fig. IV-4).

Rooting pattern was lowly heritable following one cycle of divergent selection (Fig. IV-5). Initial selections were made at the uni-

Table IV-1. Mean seedling root scores (SRS) using a 1-7 scale 1/ for the progeny of 38 TR1 clones and Ladak 65.

Clone no.	No. of plants evaluated	SRS	Clone no.	No. of plants evaluated	SRS
TR1- 2	63	2.91	TR1-54	48	3.30
TR1- 9	102	2.95	TR1-26	93	3.33
TR1- 7	98	2.99	TR1-44	33	3.36
TR1-55	27	2.99	TR1-27	106	3.38
TR1-29	111	3.00	TR1-52	35	3.41
TR1-33	99	3.03	TR1-18	58	3.44
TR1-30	103	3.04	TR1- 4	96	3.47
TR1-50	74	3.05	TR1-19	117	3.48
TR1-12	86	3.13	TR1-49	40	3.49
TR1-13	91	3.13	TR1-51	90	3.50
TR1-21	33	3.15	TR1-20	81	3.53
TR1- 8	103	3.17	TR1-56	48	3.54
TR1- 3	21	3.19	TR1-40	42	3.55
TR1-24	112	3.21	TR1-28	80	3.70
TR1-22	127	3.22	TR1-23	99	3.71
TR1-25	95	3.22	Ladak 65	130	3.71
TR1-32	82	3.22	TR1-10	53	3.73
TR1- 6	54	3.30	TR1-31	101	3.78
TR1-16	126	3.30	TR1-53	92	3.80
TR1-37	99	3.30			
			Mean of Progenies		3.30

LSD (0.05) = 0.42
CV = 11.2%

1/ 1 = tap root only, no secondary roots; 7 = tap root and numerous secondary roots.

Table IV-2. Mean seedling root scores (SRS) using a 1-7 scale 1/ for the progeny of 38 FR1 clones and Ladak 65.

Clone no.	No. of plants evaluated	SRS	Clone no.	No. of plants evaluated	SRS
FR1-45	64	4.67	FR1-39	64	3.93
FR1-40	20	4.65	FR1-11	77	3.87
FR1-38	57	4.59	FR1-13	61	3.87
FR1-42	54	4.48	FR1-25	28	3.87
FR1-29	69	4.30	FR1-41	36	3.84
FR1-55	53	4.27	FR1- 3	45	3.83
FR1-23	32	4.19	FR1-51	69	3.83
FR1-20	89	4.15	FR1-19	29	3.81
FR1-48	75	4.15	FR1-17	47	3.80
FR1- 6	68	4.13	Ladak 65	129	3.80
FR1- 2	78	4.09	FR1-22	29	3.77
FR1- 5	52	4.05	FR1- 8	60	3.73
FR1-44	30	4.04	FR1-56	40	3.70
FR1-28	47	4.02	FR1- 1	83	3.69
FR1-46	68	4.01	FR1-36	75	3.67
FR1-52	33	3.99	FR1-50	85	3.63
FR1- 7	62	3.97	FR1-32	72	3.61
FR1-54	75	3.95	FR1-53	60	3.47
FR1-10	22	3.94			
			Mean of progenies		3.98

LSD (0.05) = 0.56
CV = 11.4%

1/ 1 = tap root only, no secondary roots; 7 = tap root and numerous secondary roots.

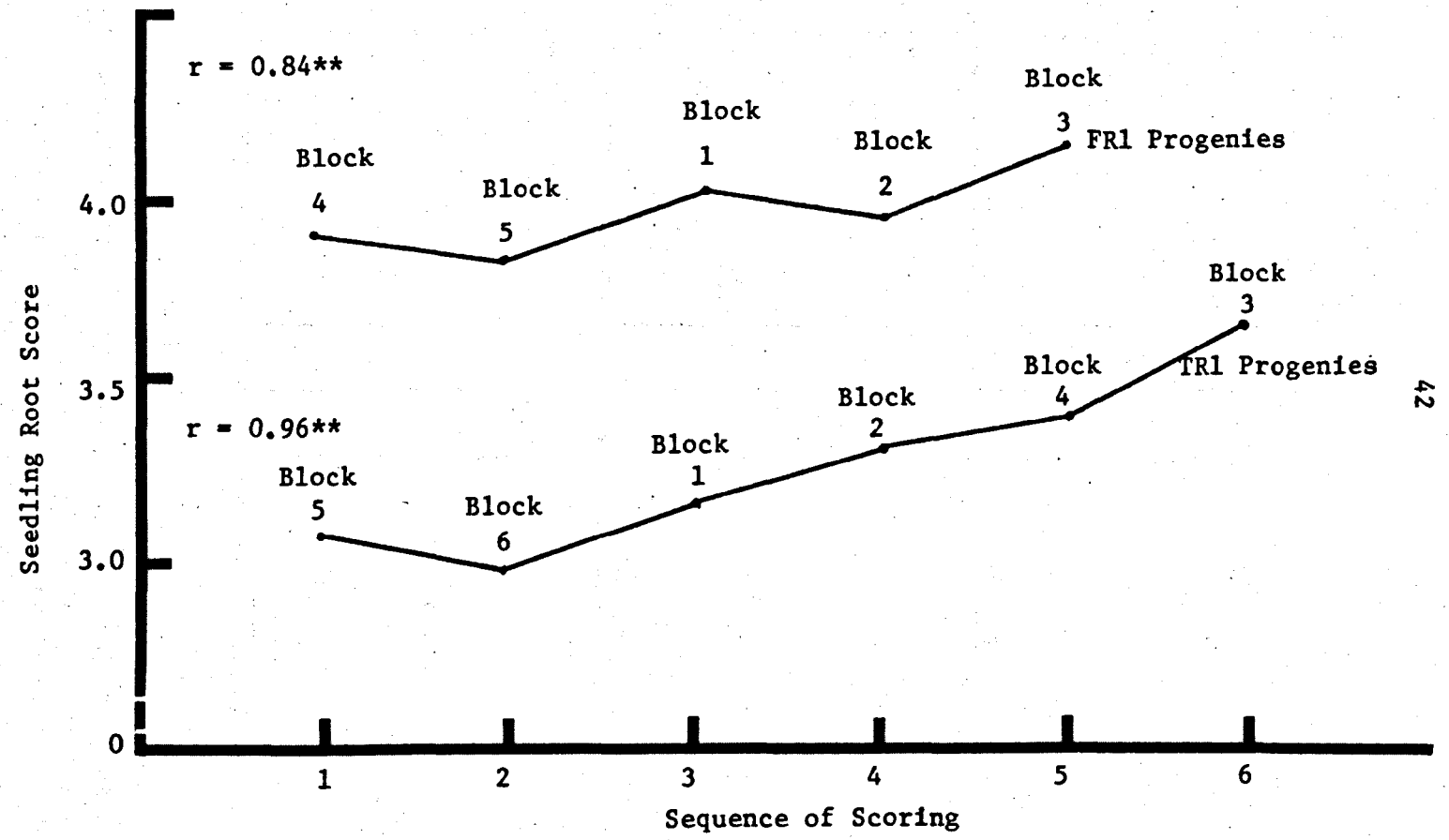

Figure IV-4. Variability in seedling root scores (1 = tap root only, no secondary roots; 7 = tap root and numerous secondary roots) as affected by sequence of scoring of the blocks.

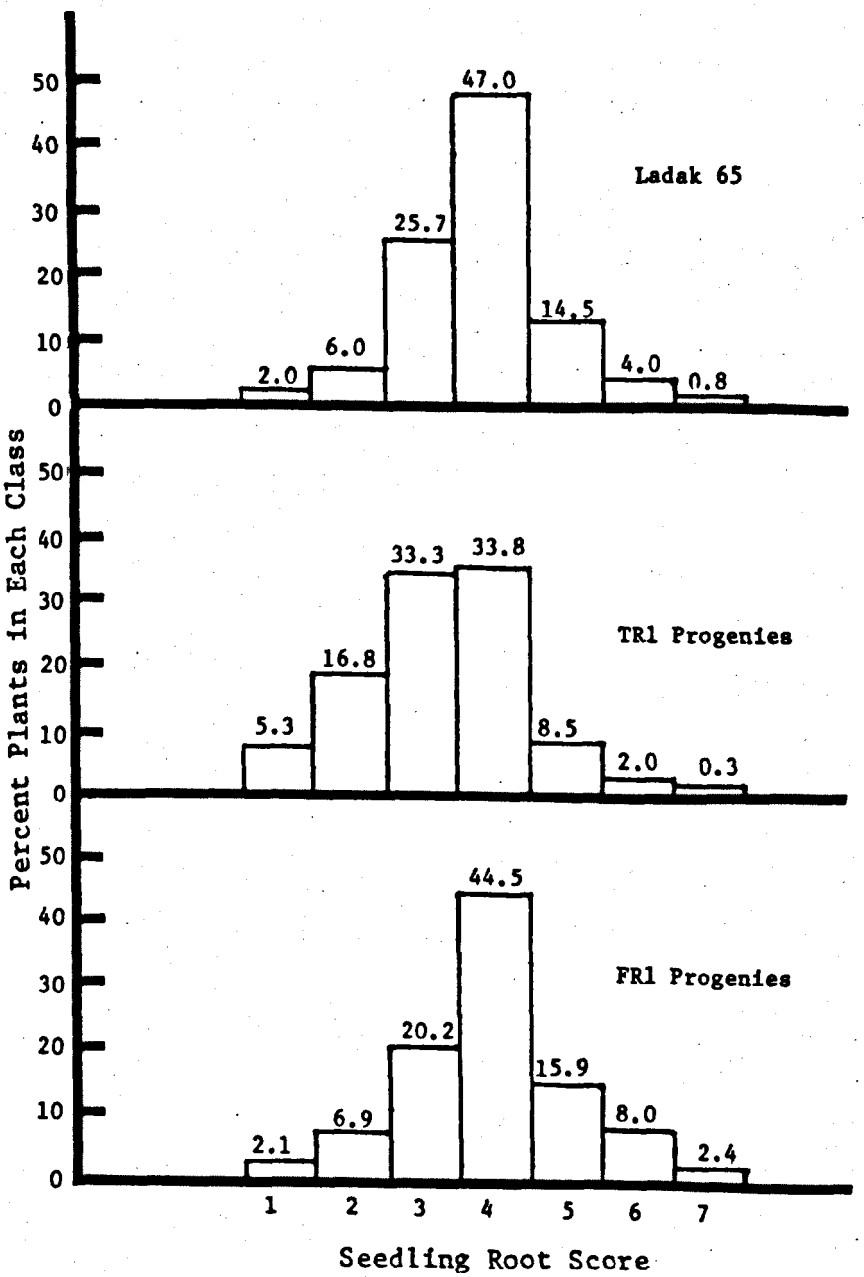

Figure IV-5. Relative frequency histograms for seedling root score (1 = tap root only, no secondary roots; 7 = tap root and numerous secondary roots) expressed as percent of plants in each class for the FR1 progenies, TR1 progenies, and Ladak 65.

foliate leaf stage and progeny evaluations were made at the first trifoliolate leaf stage. These heritability estimates may be biased due to evaluating plants at different growth stages.

Cycle Two Progeny Test

Significant differences were detected among progenies. Progenies of FR2-57, FR2-44, FR2-48, FR2-39, FR2-55, FR2-11, and FR1-45 had significantly greater seedling root scores than Ladak 65, and progenies of TR2-38, TR2-65, TR2-1, TR2-57, TR2-19, TR2-36, TRG1-12, TR2-33, TR2-13, TR2-56, TR2-6, and TR2-60 had significantly lower seedling root scores than Ladak 65 (Appendix table 2). Mean seedling root scores for TR2 (3.36) and FR2 (3.95) progenies (Table IV-3) represented an 8.4 percent and a 7.6 percent differential, respectively, from unselected Ladak 65 (3.62). Although mean seedling root scores for TR2 and FR2 progenies were not significantly different than the mean of Ladak 65, they were different from each other. The mean scores were nearly identical to the scores obtained following the first cycle of selection (Tables IV-1 and IV-2). Mean seedling root scores for the TRG1 (3.27) and FRG1 (3.93) progenies differed from each other. They represented a 10.9 percent and a 7.1 percent differential, respectively, from unselected Ladak 65. The scores were not significantly different from Ladak 65 and were similar to the scores obtained

Table IV-3. Mean seedling root scores (SRS) using a 1-7 scale 1/ of TR2, FR2, TR1, FR1, TRG1, and FRG1 progenies and unselected Ladak 65.

Population	Mean SRS*	Progenies evaluated	Plants evaluated
TR2	3.36 a	55	1,317
FR2	3.95 b	49	1,241
TR1	3.34 a	8	214
FR1	3.91 b	8	209
TRG1	3.27 a	5	95
FRG1	3.93 b	5	107
Ladak 65	3.67 ab	--	96

*Means not followed by the same letters differ at 0.05 probability level.

1/ 1 = tap root only, no secondary roots; 7 = tap root and numerous secondary roots.

following one and two cycles of phenotypic recurrent selection. Frequency histograms are shown in Figure IV-6. The heritabilities of the tap-rooted habit and the fibrous-rooted habit were both zero following the second cycle of divergent phenotypic recurrent selection.

Seven TR1 and seven FR1 progenies were evaluated in both the cycle one and cycle two progeny tests to assess the repeatability of the seedling root screening technique (Table IV-4). The correlation among these tests for all progenies was 0.89**. Spearman rank correlations for the TR1 and FR1 progenies were 0.87** and 0.96**, respectively. Thus, even though seedling root score may vary from test to test, the relative rankings of the progenies remained the same.

Following two complete cycles of divergent phenotypic recurrent selection the seedling root screening method is repeatable but little progress was realized from selection (Fig. IV-6). Following one cycle of phenotypic recurrent selection no progress was made either through an additional phenotypic selection cycle or by intercrossing superior clones on the basis of the cycle one progeny test. Another selection cycle is needed to determine if further progress can be made.

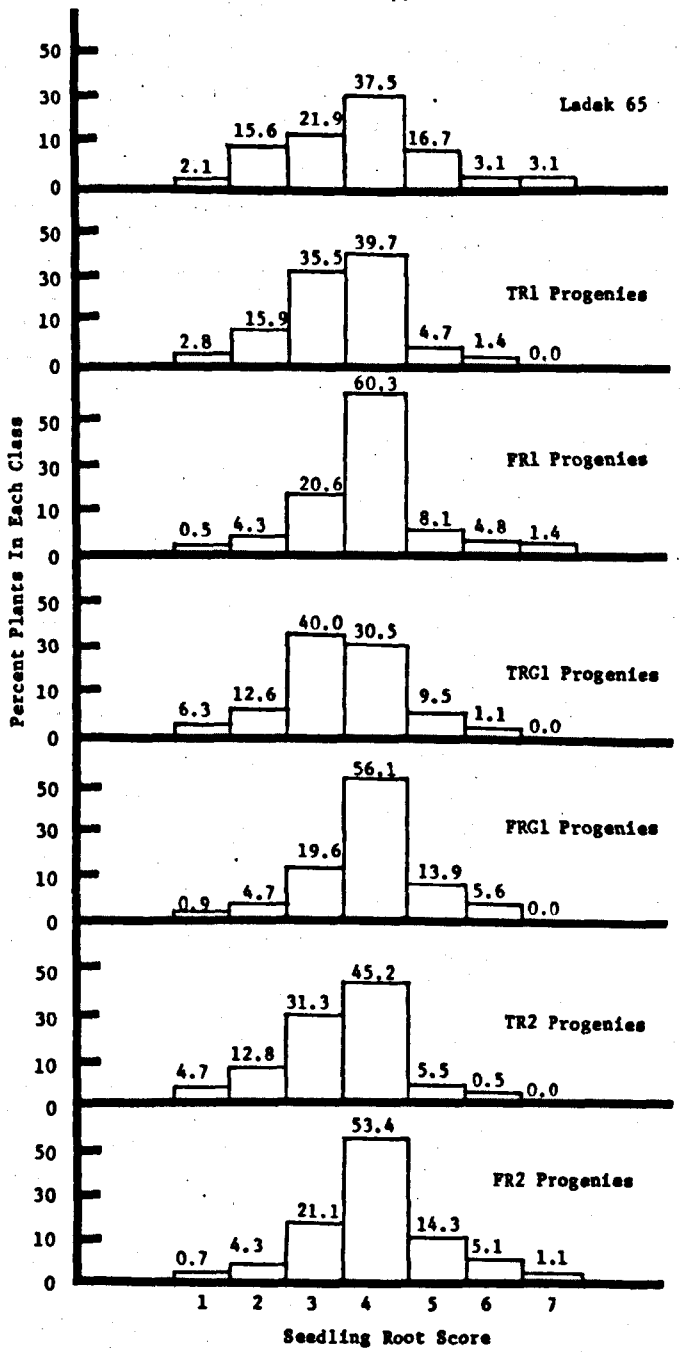

Figure IV-6. Relative frequency histograms for seedling root score (1 = tap root only, no secondary roots; 7 = tap root and numerous secondary roots) expressed as percent of plants in each class for the FR2 progenies, TR2 progenies, FRG1 progenies, TRG1 progenies, FR1 progenies, TR1 progenies, and Ladak 65.

Table IV-4. Comparison of seedling root scores (SRS) using a 1-7 scale 1/ for several TR1 and FR1 progenies and Ladak 65 in the cycle one (1) and cycle two (2) progeny tests.

Progeny	SRS(1)	SRS(2)
TR1- 2	2.91	3.07
TR1- 9	2.95	3.04
TR1-29	3.00	3.29
TR1-30	3.04	3.47
TR1-12	3.13	3.29
TR1- 8	3.17	3.68
TR1-24	3.21	3.58
FR1-28	4.02	3.73
FR1-14	4.07	3.52
FR1- 2	4.09	3.75
FR1-20	4.15	3.92
FR1-29	4.30	4.02
FR1-42	4.48	4.22
FR1-45	4.67	4.35
Ladak 65	3.75	3.67

1/ 1 = tap root only, no secondary roots; 7 = tap root and numerous secondary roots.

Cultivar and Germplasm Survey

Sixteen germplasms and 10 commercial cultivars were assayed for seedling root score. Highly significant differences were detected among entries (Table IV-5). Falcata composite and many of the cultivars possessing a M. falcata background such as 'Rhizoma', 'Drylander', 'Orenburg', 'Kane', and 'Ranger' all had relatively lower seedling root scores indicating a strong tendency toward a tap-rooted habit. Ladak 65, though, which had a seedling root score of 3.78, was intermediate in rooting habit but exceeded by only one cultivar, Thor. The heat resistant types (HR1), selected from Ladak 65 and most of the other cultivars and some germplasms. Plants selected for heat resistance were able to regrow from the crown after being subjected to 50 C for 12 hours in a drying oven. Thus, seedling survival of heat-stressed plants may be related to the fibrosity of their root system.

Significant differences for seedling root score were not detected between prostrate (PR1) and erect (ER1) types, regrowth (MR1) and non-regrowth (MN1) types, wilt susceptible (WS1) and wilt resistant (WR1) types, large leaflet (LG1) and small leaflet (SM1) types, and pale leaf (PA1) and dark leaf (DK1) types indicating that selection for these traits does not affect the rooting habit. Many of these germ-

Table IV-5. Mean seedling root scores (SRS) using a 1-7 scale 1/ for 16 germplasm and 10 commercial cultivars.

Germplasm or cultivar	Number of plants evaluated	Mean SRS
Heat resistant types (HR1)	98	4.30
Low photorespiring progeny ("C-4")	93	4.27
Prostrate types (PR1)	99	4.27
Pale leaf types (PA1)	99	4.13
Nonregrowth types (MN1)	97	4.10
Erect growth types (ER1)	90	4.07
Regrowth types (MR1)	98	4.00
Wilt susceptible types (WS1)	104	3.96
Large leaf types (LG1)	103	3.95
Thor	105	3.95
Small leaf types (SM1)	100	3.90
Wilt resistant types (WR1)	95	3.88
Dark leaf types (DK1)	102	3.80
Anchor	103	3.78
Ladak 65	105	3.78
Grimm	102	3.74
Early maturing types (EM1)	85	3.70
C-6 germplasm pool	104	3.67
Orenburg	92	3.63
C-3 germplasm pool	98	3.62
Vernal	96	3.60
Rhizoma	106	3.58
Drylander	102	3.57
Kane	98	3.54
Ranger	108	3.49
Falcata composite	105	3.20

LSD (0.05) = 0.36
CV = 6.7%

plasms were again tested during the cycle two progeny test and similar results were again obtained (Appendix table 3) indicating the repeatability of the trait.

Considerable variability exists for the seedling root trait and exploitation of several germplasm pools will most likely raise or, conversely, lower seedling root scores beyond that already realized by drought selection within Ladak 65.

CHAPTER V

DIVERGENT SELECTION FOR RESISTANCE TO WILTING USING CONETAINERS

Some alfalfa seedlings wilt early when exposed to drought conditions and thus may avoid drought through reduction in exposed surface. Other seedlings remain turgid throughout a dry period thus resisting water stress. Divergent phenotypic selection within Ladak 65 was used to determine if progress for these traits could be made and to determine their heritabilities.

Materials and Methods

Preliminary Study

On October 20, 1977, 18 conetainers (66 cm^3 pine cells) were filled with no. 3 terralite. The open bottoms were covered with cheesecloth held in place by strapping tape. Ladak 65 was planted 1.3 cm deep in nine conetainers and nine others remained without seed. The conetainers, in a conetainer rack, were placed in a growth chamber that had fans and a 12-hour diurnally alternating 21-24 C temperature and 16,000 lux light intensity. All conetainers were alternately watered with tap and nutrient (2.3 g N, 2.3 g P_2O_5, 2.3 g K_2O, and 18.9 ℓ H_2O) water four times weekly.

After 50 days, when all the plants had reached the fifth trifoliolate leaf stage, the conetainers were subirrigated for 12 hours to saturate the terralite, and then allowed to dry for 158 hours. The conetainers were weighed periodically during the drying period to moni-

tor water loss. Data recorded included time of initial wilt symptoms (top leaf wilted) during the drying period, dry weight of tops and roots at initial wilt, and weight of the terralite and empty conetainers at the end of the experiment. The terralite was dried in an oven at 82 C for 24 hours before weighing. Water content of each conetainer was estimated from these data at various times during the drying period.

Initial Selections

Number 3 terralite was ground in a soil grinder and mixed thoroughly in a cement mixer to obtain samples of uniform density. One thousand conetainers were filled with ground terralite and weighed. Ladak 65 seeds remaining on a 0.13 cm wire mesh screen were planted 1.3 cm deep in the conetainers and the seedlings were grown as in the preliminary study.

The seedlings were tested in three groups of approximately 275 plants each. At the second trifoliolate leaf stage the terralite in these conetainers was saturated and dried as in the preliminary study. Plants not at this stage of development were discarded to insure uniformity.

Plants which were the first to wilt in each group as well as those which wilted last were saved. Plants were considered wilted when the top leaves of the plant appeared to wilt. A random sample

of plants were weighed periodically during the drying period in each of the three groups to monitor water loss. Plants saved comprised the cycle one wilt-susceptible (WS1) and cycle one wilt-resistant (WR1) populations. Each population was separately intercrossed in the greenhouse beeroom and the seed from each plant was individually harvested, cleaned, and packeted. Repeated periods of intercrossing were needed to obtain enough seed from each plant for a progeny test.

Progeny Test

Progenies of the wilt-susceptible and the wilt-resistant plants were tested in a growth chamber at 21 C and a 12-hour photoperiod. The wilt-susceptible and the wilt-resistant progenies were tested in separate experiments using a randomized complete-block design with three replications and seven plants per replication. Procedures used were identical to those used for the initial population screening, except the seeds were not sized. Data recorded included initial weights of all the conetainers with terralite, the day the plant displayed initial wilt symptoms, weights of the conetainers when the plant wilted, moisture content of the terralite at wilt, shoot and root wet weight and dry weight, percent plant moisture at wilt, and root to shoot ratios. Moisture content of the terralite was estimated by subtracting the initial weight of the conetainer containing dry

terralite and the wet plant weight at wilt from the weight of the conetainer, terralite, and plant at wilt. Wet weight measurements were taken immediately after the plant wilted. Dry weights were recorded after the plants had dried for 7 days in an oven at 82 C. Selected plants could not be weighed since this would have impaired survival. Harvey's least squares analysis was used to analyze variables, since plant numbers in each replicate varied due to differential survival and discarding plants not at the second trifoliolate leaf stage at the beginning of the drydown period.

Results and Discussion

Preliminary Study

Considerable variability was observed for rate of water loss from the conetainers (Table V-1). Rates of water loss from conetainers without plants were less variable and had decreased total water loss (Fig. V-1). The most striking difference in rate of water loss occurred between 0 and 25 hours when conetainers with plants dried at an average of 0.63 g/hr whereas those without plants dried at 0.26 g/hr. After 25 hours the rate of water loss was similar for conetainers whether with or without plants. Transpiration increased the rate of water loss substantially early in the drying period.

All the plants showed initial wilt symptoms by 145 hours (Table V-2). Plants having the most topgrowth were the first to wilt and those with

Table V-1. Average rates of water loss (g/hr) from conetainers containing terralite with and without plants.

Cone no.	Time sequence after saturation (hrs) 0-25	25-54	54-74	74-101	101-122	122-158
Without plants						
1	0.26	0.23	0.22	0.19	0.09	0.14
2	0.28	0.26	0.20	0.12	0.07	0.10
3	0.24	0.20	0.15	0.10	0.07	0.12
4	0.29	0.27	0.24	0.18	0.08	0.13
5	0.29	0.19	0.14	0.11	0.08	0.12
6	0.28	0.26	0.26	0.17	0.08	0.10
7	0.22	0.20	0.21	0.18	0.09	0.08
8	0.30	0.25	0.21	0.15	0.07	0.09
9	0.22	0.22	0.22	0.17	0.09	0.12
Mean and Std. Error	0.26 ±0.009	0.23 ±0.009	0.21 ±0.012	0.15 ±0.012	0.08 ±0.003	0.11 ±0.006
With plants						
1	0.53	0.23	0.33	0.20	0.09	0.08
2	0.79	0.26	0.21	0.10	0.04	0.03
3	0.49	0.20	0.25	0.15	0.08	0.09
4	0.78	0.27	0.21	0.11	0.06	0.05
5	0.70	0.19	0.26	0.13	0.06	0.06
6	0.38	0.26	0.27	0.17	0.08	0.11
7	0.79	0.20	0.15	0.08	0.03	0.02
8	0.77	0.25	0.25	0.12	0.06	0.05
9	0.40	0.22	0.31	0.21	0.14	0.12
Mean and Std. Error	0.63 ±0.057	0.23 ±0.009	0.25 ±0.018	0.14 ±0.015	0.07 ±0.012	0.07 ±0.012

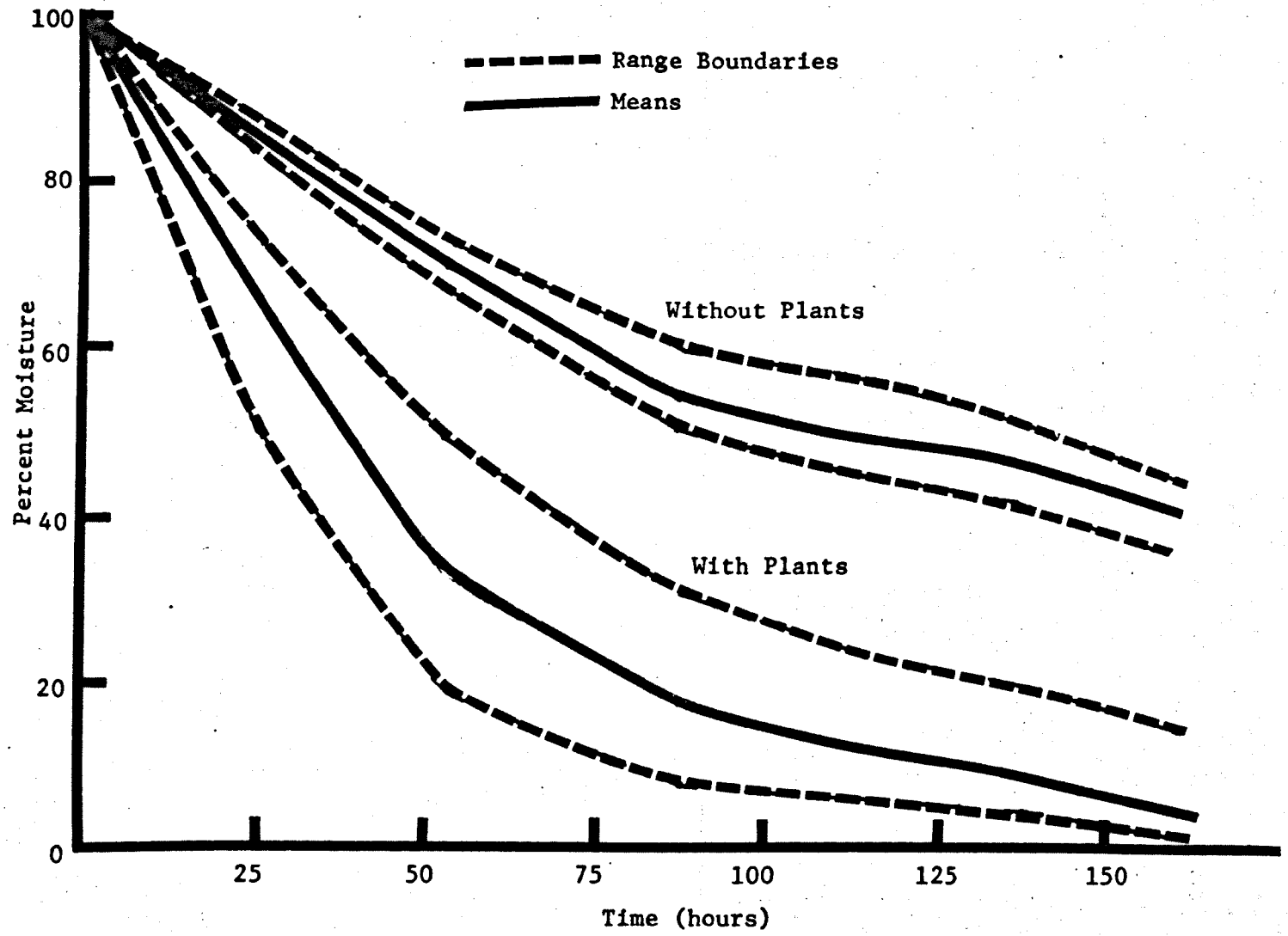

Figure V-1. Comparison of percent water loss (mean and range) expressed as percent of total water present at saturation during the drying period among conetainers with and without alfalfa seedlings.

Table V-2. Approximate hour of expression of initial wilt symptoms, percent of total initial moisture remaining in the terralite at wilt, dry weights of tops and roots, and root to shoot ratios for nine Ladak 65 alfalfa seedlings drying in conetainers.

Conetainer no.	Hour of wilt	Percent H_2O in terralite at wilt	Top weight	Root weight	Root/Shoot
7	77	7.2	0.21	0.15	0.71
2	88	7.6	0.20	0.13	0.65
4	88	10.8	0.16	0.11	0.69
8	92	10.2	0.21	0.15	0.71
5	117	7.5	0.16	0.08	0.50
9	117	19.5	0.14	0.08	0.57
1	134	7.2	0.14	0.10	0.71
3	134	14.1	0.12	0.14	1.17
6	145	16.8	0.04	0.06	1.50

the least wilted last. The correlation between top dry weight and wilting time was -0.87**. Conetainers with plants that wilted at 88 hours lost water at the rate of 0.79 g/hr versus those with plants which wilted after 122 hours at 0.47 g/hr (Fig. V-2). The correlation between rate of water loss between 0 and 25 hours and top dry weight was 0.85**, whereas between 122 and 158 hours the correlation was -0.81**. Plants with relatively more topgrowth lose water more rapidly early in the drying period. Later in the drying period, as these larger plants continue to wilt, they lose water less rapidly than smaller plants that have not wilted.

Root to shoot ratios (dry weight basis) averaged 0.68 for plants which wilted by 88 hours and 1.13 for plants which wilted after 122 hours. The correlation between root to shoot ratio (dry weight basis) and time of wilt was 0.62** indicating that plants with a higher proportion of root growth are able to resist wilting for a longer period.

Variability of drying rates for conetainers without plants indicates that more uniformity is needed if this method is to be employed to screen plants. Terralite particle size and density should be as uniform as possible from conetainer to conetainer. Since top dry weight is correlated with wilting time, it is imperative to screen plants at the same growth stage. This might enable selection of plants which do not merely avoid water loss through reduced topgrowth, but are truly

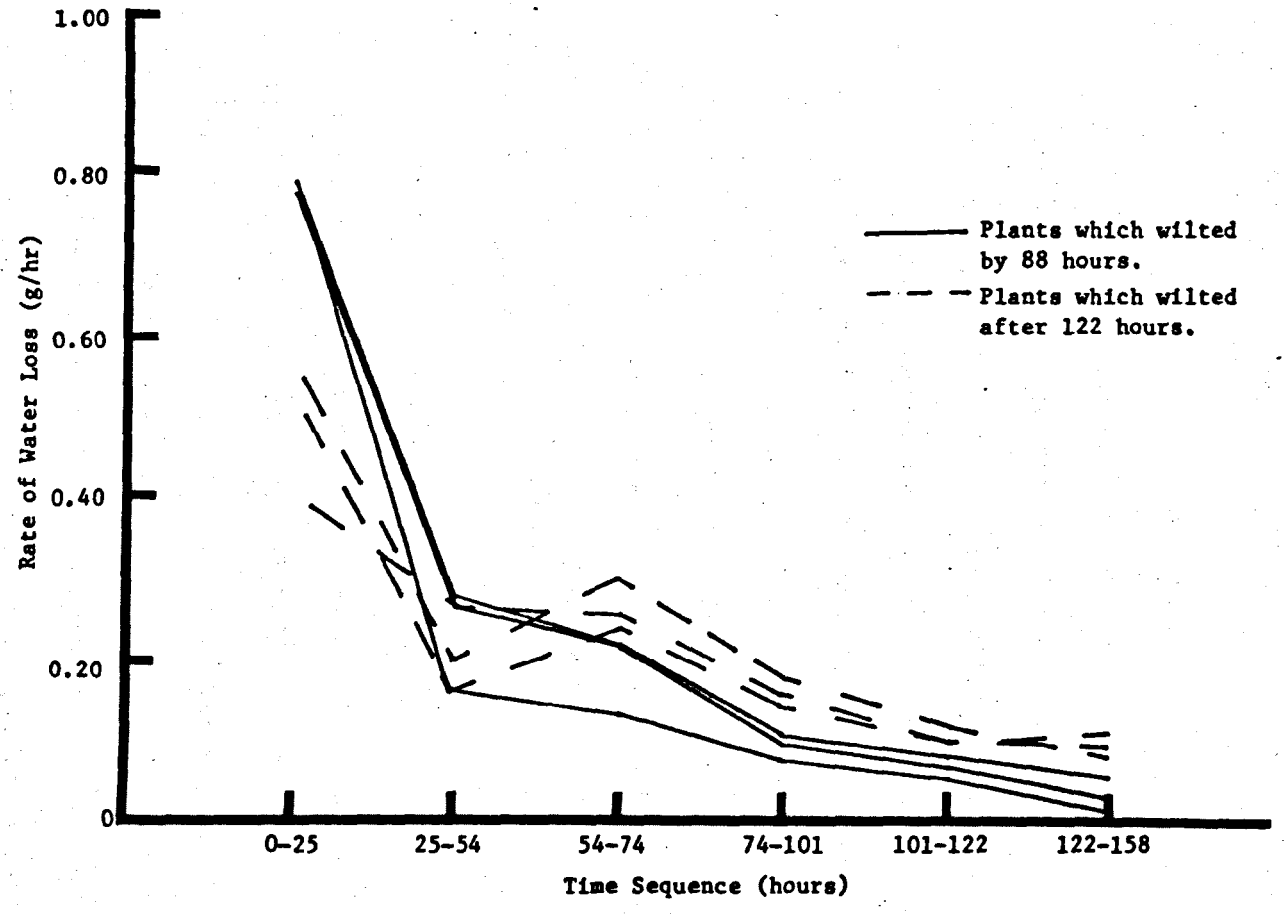

Figure V-2. Relative rates of water loss for alfalfa seedlings in conetainers containing terralite which wilted by 88 hours compared to those which wilted after 122 hours.

desiccation tolerant. Initiating the drying period when all the plants are at an earlier growth stage might insure better uniformity.

Initial Selections

Plants (834) were screened for resistance to wilting in three groups of successive times to insure similar developmental growth stages. Both early-wilting plants (WS1) and late-wilting plants (WR1) were saved. The selection intensities for the WS1 and WR1 populations were 8.3 (69 plants) and 8.5 (71 plants) percent, respectively. The distribution was skewed toward the early-wilting types (Fig. V-3).

Drying rates were very similar during each of the three runs monitored (Table V-3). Thus, using ground and mixed terralite and sizing plants prior to drydown reduced variability and probably enhanced selection of superior genotypes.

Progeny Test

Twenty-five WS1 progenies were tested with four WR1 progenies, composited TR1 and FR1 progenies, and Ladak 65 as a check. The first plant wilted 36 days following the start of the drying period. The drying period extended for another 41 days and 27 plants never wilted. Significant differences were detected among progenies for day of wilt (Appendix table 4). After 60 days of drying, 50.3 percent of the WS1 plants had wilted, whereas only 38.5 percent of the Ladak 65 plants had wilted (Fig. V-4). After 76 days of drying, only 4.6 percent of the

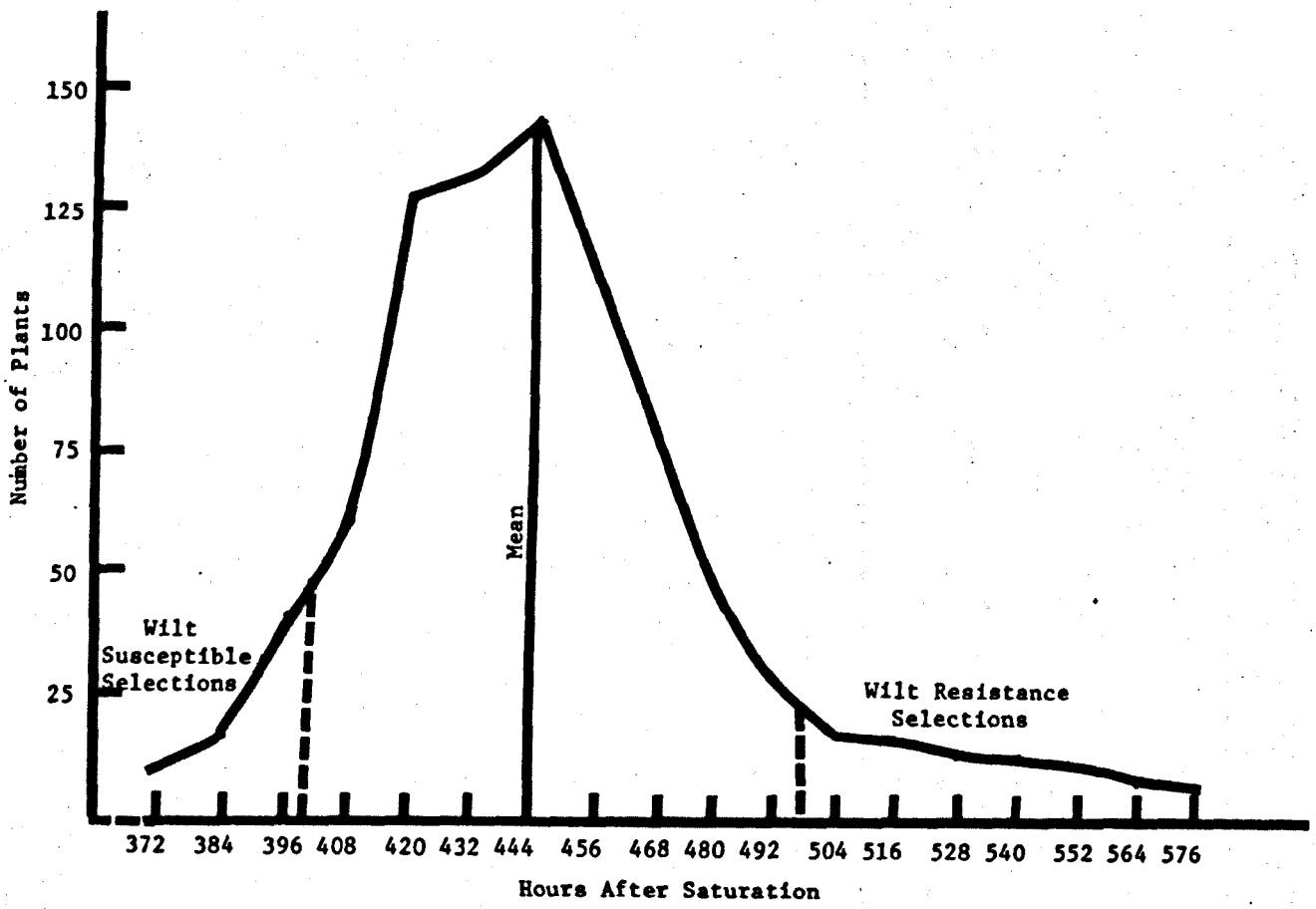

Figure V-3. Frequency distribution for wilting time for the initial screening of Ladak 65 alfalfa seedlings drying in conetainers containing terralite.

Table V-3. Average rates of water loss (g/hr) during successive time intervals during the drying period for the initial screening of Ladak 65 alfalfa seedlings using conetainers.

	Time intervals (hrs) during drying period					
Group	0-96	96-192	192-288	288-384	384-480	480-576
1	0.25	0.19	0.16	0.09	0.01	0.01
2	0.26	0.20	0.16	0.08	0.01	0.01
3	0.25	0.19	0.15	0.10	0.02	0.01
Mean and Std. Error	0.25 ±0.003	0.19 ±0.003	0.16 ±0.003	0.09 ±0.005	0.01 ±0.003	0.01 ±0.000

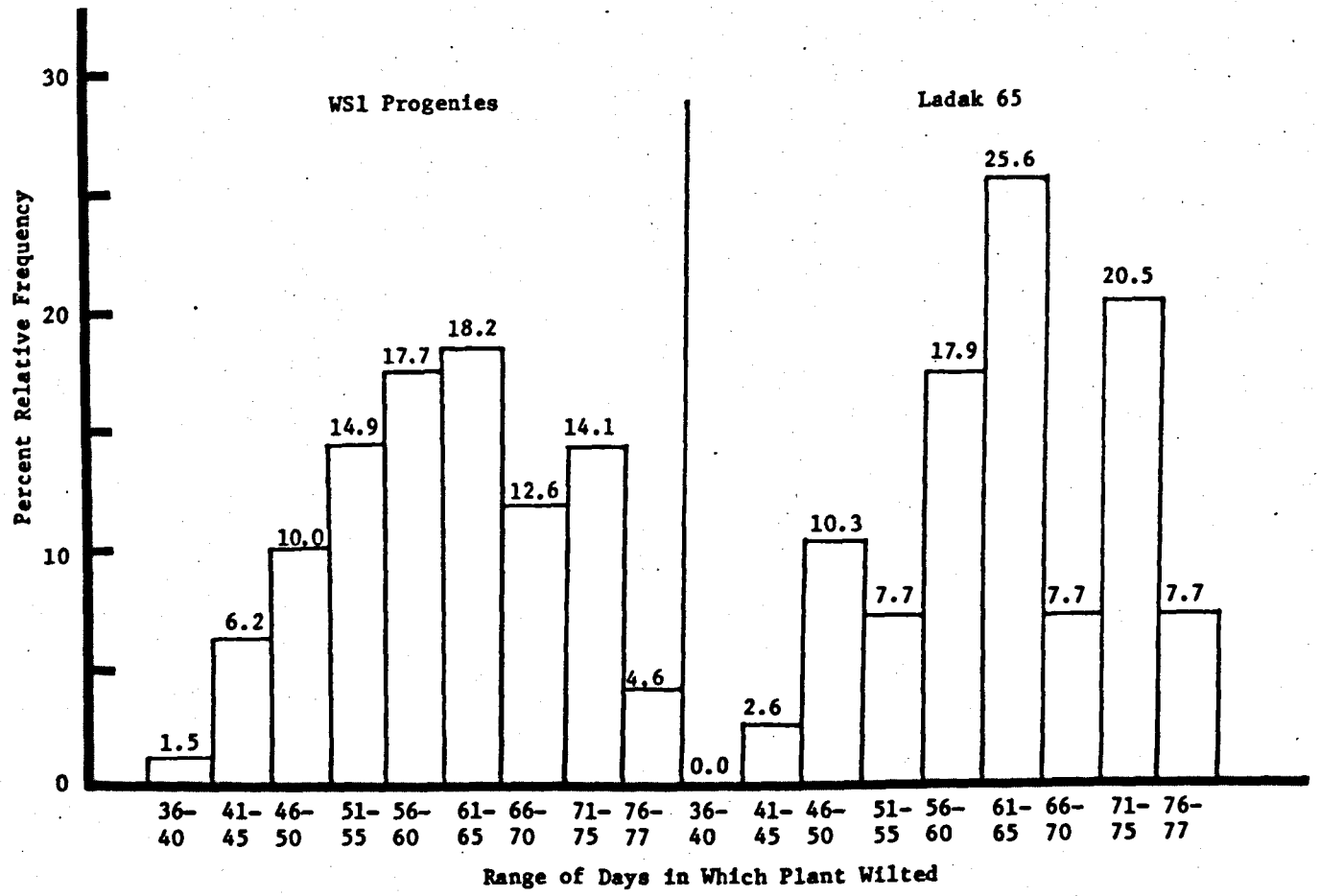

Figure V-4. Relative frequency distributions for mean day of wilt for individual plants from 25 WS1 progenies and Ladak 65 drying in conetainers containing terralite.

WS1 plants remained unwilted whereas 7.7 percent of the Ladak 65 plants had not wilted. Two progenies, WS1-11 and WS1-15, wilted significantly earlier than Ladak 65 (Table V-4). The mean wilting time of all WS1 progenies was 59.9 days, significantly lower than the mean of the Ladak 65 plants tested (62.9 days). The TR1 plants tested also wilted earlier than Ladak 65 indicating a possible relationship between a tap-rooted habit and early wilt.

Thirty WR1 progenies were tested with two WS1 progenies and Ladak 65 as a check. The first plant wilted 38 days following the start of the drying period. The drying period extended for another 23 days and 103 plants had not yet wilted. These plants were saved to use as the source population for further selection cycles.

Significant differences were detected among progenies for days to wilt (Table V-5). After 51 days of drying, 26.1 percent of the WR1 plants had wilted whereas 28.2 percent of the Ladak 65 plants had wilted (Fig. V-5). After 60 days, 17.7 percent of the WR1 plants had not wilted whereas 11.3 percent of the Ladak 65 plants remained unwilted. Five WR1 progenies, WR1-5, WR1-27, WR1-38, WR1-41 and WR1-44, wilted significantly later than Ladak 65. The mean wilting time for all WR1 progenies was 54.5 days, significantly higher than the mean of the Ladak 65 plants tested (53.5 days).

Correlations between day of wilt and several variables were determined for both WS1 and WR1 progenies (Table V-6). Since plant weights

Table V-4. Mean day of wilt for 25 WS1 progenies, 4 WR1 progenies, Ladak 65, TR1 plants, and FR1 plants after drying in containers containing terralite in a growth chamber.

Progeny	No. of plants	Mean	Variance
WS1-15	17	54.9	135.06
WS1-11	16	55.1	90.25
WS1-43	14	56.4	98.57
WS1-21	18	56.9	136.88
WS1-23	18	57.3	82.59
WS1-39	18	57.4	50.85
WS1-35	16	57.6	47.85
WS1-18	14	58.6	68.42
WS1-33	15	58.7	91.09
WS1- 1	12	59.0	68.91
WS1- 8	16	59.2	68.33
WS1-27	11	59.3	94.02
WS1-10	14	59.6	178.88
WS1- 9	18	60.7	94.33
WS1-34	16	60.7	99.27
WS1-14	12	61.1	73.36
WS1-42	15	61.8	85.17
WS1-26	13	62.1	150.41
WS1-30	20	62.1	74.52
WS1-31	12	62.1	112.08
WS1-41	16	62.2	130.70
WS1- 2	15	62.3	75.92
WS1-12	16	62.7	145.96
WS1-52	16	64.4	55.06
WS1- 4	13	65.2	45.03
Mean		59.9	
WR1-18	15	57.1	57.07
WR1- 5	15	61.1	70.92
WR1-57	21	62.9	62.95
WR1-27	16	68.6	46.53
Ladak 65	37	62.9	62.92
TR1	16	54.6	146.40
FR1	17	62.0	91.37
LSD (0.05)		6.7	
CV%		16.2	

Table V-5. Mean day of wilt for 30 WR1 progenies, 2 WS1 progenies, and Ladak 65 after drying in conetainers containing terralite in a growth chamber.

Progeny	No. of plants	Mean	Variance
WR1-12	20	48.5	41.73
WR1- 8	17	50.5	39.14
WR1- 9	19	50.6	55.13
WR1-10	19	50.6	51.25
WR1- 4	16	51.1	32.92
WR1- 1	19	52.0	43.16
WR1-39	16	52.1	30.20
WR1-13	16	52.6	43.32
WR1-32	19	53.0	23.22
WR1-15	18	53.8	19.91
WR1-29	18	53.9	30.61
WR1-36	18	54.1	30.93
WR1-24	18	54.7	36.35
WR1-28	17	55.1	37.31
WR1-57	19	55.2	18.06
WR1-37	20	55.3	18.77
WR1- 2	14	55.4	23.63
WR1-31	18	55.4	11.43
WR1-11	18	55.6	33.66
WR1-30	20	55.7	32.72
WR1-42	19	55.9	18.83
WR1-34	18	56.4	31.66
WR1-40	19	56.4	39.48
WR1-26	18	56.5	24.62
WR1-43	19	56.5	35.93
WR1-41	18	56.6	16.72
WR1-44	20	57.0	15.58
WR1-27	19	57.3	14.43
WR1-38	18	58.1	16.41
WR1- 5	13	58.3	4.56
Mean		54.5	
WS1-11	19	48.4	15.13
WS1-15	20	54.2	26.20
Ladak 65	53	53.5	27.75
LSD (0.05)		3.1	
CV%		8.9	

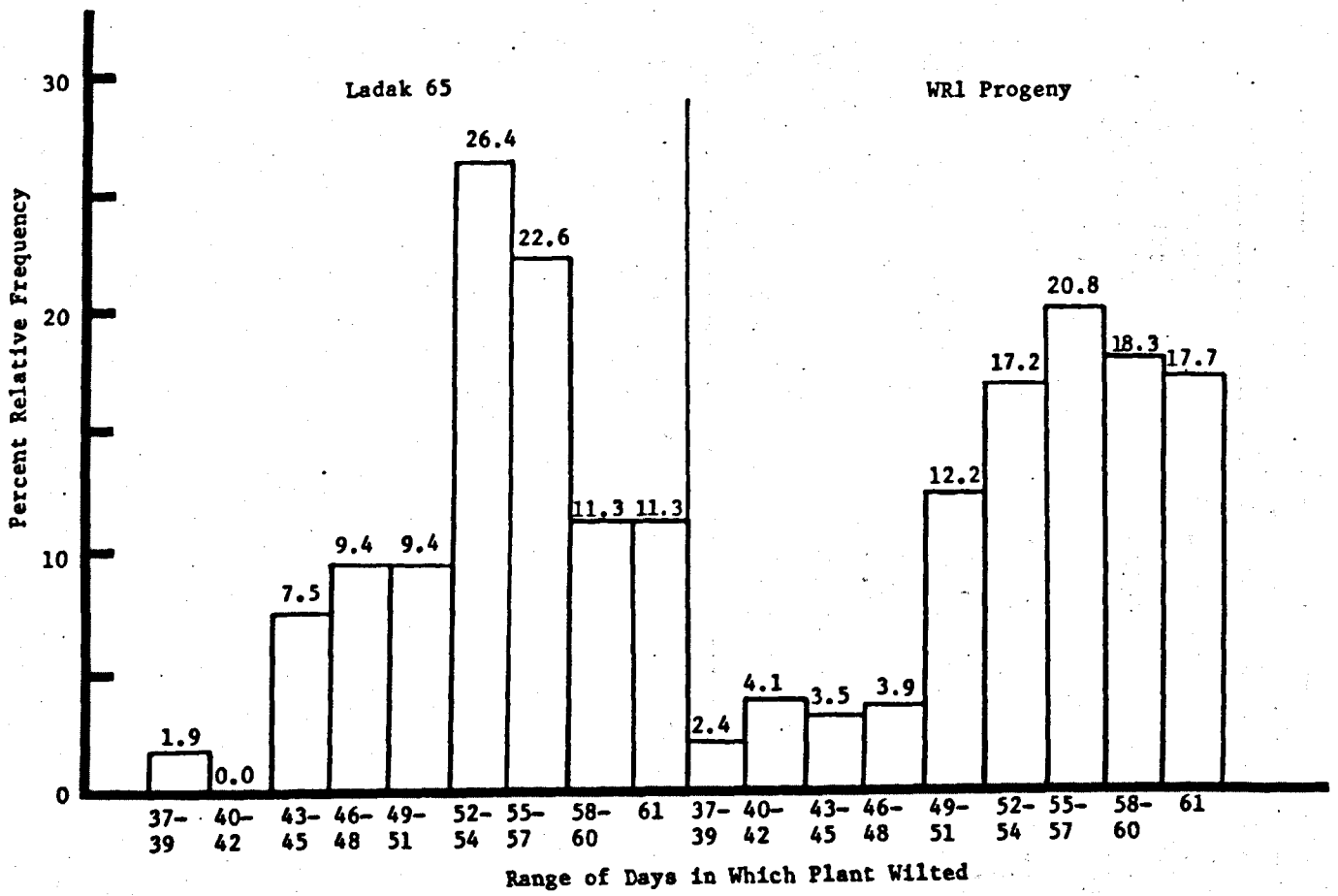

Figure V-5. Relative frequency distributions for mean day of wilt for individual plants from 30 WR1 progenies and Ladak 65 drying in conetainers containing terralite.

Table V-6. Correlations between several variables and day of wilt for the WS1 and the WR1 progenies.

Variable	r value	
	WS1 progenies	WR1 progenies
Wet shoot weight at wilt	-0.441**	-0.716**
Wet root weight at wilt	-0.573**	-0.166**
Wet plant weight at wilt	-0.621**	-0.672**
Wet root/shoot	-0.179	0.397**
Dry shoot weight	-0.164	-0.627**
Dry root weight	-0.118	-0.161**
Dry plant weight	-0.172	-0.552**
Dry root/shoot	-0.031	0.336**
% H_2O in shoot	-0.380**	-0.381**
% H_2O in root	-0.524**	-0.070
Weight of terralite at wilt	-0.391*	-0.141**

*,** significant at the .05 and .01 levels, respectively.

were not recorded for selected plants these correlations could be misleading if not carefully interpreted. Significant to highly significant negative correlations were detected for day of wilt and wet shoot weight, wet root weight, wet plant weight, percent moisture in the shoot, and moisture content of the terralite at wilt. Plants which weighed the most at wilt tended to wilt earlier. These early wilting plants also had a relatively higher percent moisture in the shoots and more moisture in the terralite. Since there was more moisture in the terralite of early-wilting plants at wilt than late-wilting plants at wilt, it appears that these early-wilting plants are probably truly wilt susceptible.

Wet and dry root to shoot ratios of the WR1 progenies were positively correlated to day of wilt, and dry root, shoot, and total plant weights were negatively correlated to day of wilt. Since the larger, early-wilting plants were saved (and thus not weighed) in the test of the WS1 progenies, these variables were not correlated with day of wilt in this test. The correlations again corroborate findings from the preliminary study that seedlings with more topgrowth and those with small root to shoot ratios wilt earlier. Since day of wilt was negatively correlated to plant weight, selection of plants which wilt later would then result in a smaller proportion of relatively large, vigorous seedlings in the selected population.

Realized heritability (9) of time of wilt for early wilt is 20.7 percent and for late wilt is 10.4 percent. Neither trait appears to be highly heritable.

To utilize this screening technique additional technique refinement is required. The technique is not a rapid one, but when weights are not recorded and daily monitoring during the drydown period is not required, this technique becomes less tedious and time-consuming. Care must be exercised in selection, so that small plants which avoid water loss are not the only types selected. Selection among the larger plants might better reverse this trend. Once size differences are minimized, and technique is improved, plants which wilt the latest should be desiccation-tolerant. The only way to prove the merit of this technique, once refined, is to determine how well plant performance in the growth chamber corresponds to drought resistance in the field.

CHAPTER VI

DIVERGENT SELECTION FOR LEAF COLOR AND LEAFLET SIZE IN ALFALFA

Pale-leaved barley plants possess lower canopy temperatures, higher albedos, higher net radiation, and greater sensible heat loss than dark-leaved barley plants (2, 81, 82). Dark-leaved barley plants have higher water-use efficiency and generally yield more. Plants with reduced leaf area are believed to possess better drought resistance (11, 231, 267, 268, 298). Large-leaved plants usually yield more due to increased photosynthetic surface. Variability exists for these traits in alfalfa and could be utilized, if heritable, in a breeding program for drought resistance. This study was conducted to determine if progress through selection could be made for pale versus dark leaf color and large versus small leaflet size in alfalfa.

Materials and Methods

Selection for Leaf Color

In August, 1977, initial selections were visually made (50 each) at Bozeman, Montana for dark (blue-green) and pale (yellow-green) leaf color. Germplasm sources for these selections included several winterhardy plant introductions from the World Collection of Alfalfa, C-3 alfalfa germplasm release, and a dryland variety trial consisting of lines from many sources. Ten cuttings were taken from each selected plant in the field and maintained in the greenhouse. In May, 1978

they were transplanted into two separate, spatially-isolated intercross blocks at Huntley, Montana for seed increase.

Seed was harvested from individual plants in each intercross block in October, 1978. Enough seed was available from 30 dark-leaved plants and 21 pale-leaved plants to plant a replicated field progeny trial at Bozeman, Montana (Bozeman silt loam soil) in May, 1979. One-hundred and fifty mechanically-scarified (Forsberg scarifier containing sandpaper) seeds were planted in each of four blocks for each clone using a randomized complete-block design. The single-row plots were spaced 60 cm apart and were 6 m long. Five commercial alfalfa cultivars, Thor, Anchor, Ladak 65, Ranger, and Vernal, were also seeded similarly as unslected checks.

The progeny were rated using a visual color rating (VCR) on a 1-5 scale (1 = pale yellow-green, 2 = pale green, 3 = intermediate green, 4 = dark green, and 5 = dark blue-green) when the plants reached 100 percent bloom in both 1979 and 1980. The plants were not under water stress as they had been irrigated and supplemented twice by rainfall prior to evaluation. Between 10 and 15 random plants were scored for each progeny row in 1979 and five random plants per progeny row were scored in 1980. A Munsell color chart (MS) was also used to devise a 1-3 color scale (1 = 5GY5/8, 2 = 5G4/6, and 3 = 2.5G3/4) to score the regrowth at the bud stage in 1979 and first growth at 100 percent bloom in 1980. Five random plants were scored in every progeny row in each of

the four blocks in both 1979 and 1980. Regrowth scores (RS) were also recorded for each progeny row at the first bud stage in 1979. The length of the longest stem was measured and rated on a 1-5 scale (1 = 0-10 cm, 2 = 10-20 cm, 3 = 20-30 cm, 4 = 30-40 cm, and 5 = 40 cm and above).

Visual color ratings were analyzed using an analysis of variance on the means. Pearson and Spearman rank correlations were used to determine how well progeny means correlated across environments and under different rating systems.

Selection for Leaflet Size

In August, 1977, initial selections (50 of each) were visually made at Bozeman, Montana for large and small leaflet size from the same germplasm sources used for the leaf color selections. Ten cuttings were taken from each plant in the field and maintained in the greenhouse. In May, 1978 they were transplanted into two separate, spatially-isolated intercross blocks at Huntley, Montana for seed increase.

Seed was harvested from individual plants in each intercross block in October, 1978. Adequate seed was available from 30 large-leaved clones and 19 small-leaved clones to plant a replicated progeny trial at Bozeman, Montana (Bozeman silt loam soil) in May, 1979. One-hundred and fifty mechanically-scarified (Forsberg scarifier containing sandpaper) seeds were planted in each of four blocks for each clone using a randomized complete-block design. The single-row plots were spaced 60 cm

apart and were 6 m long. Five commercial alfalfa cultivars, Thor, Anchor, Ladak 65, Ranger, and Vernal, were seeded as checks.

Leaflet size was measured from lateral leaflets from five random stems per progeny row in each block in 1979 using a leaf area meter (LAM) in the laboratory. The plants were at the 100 percent bloom stage and only leaflets from leaves directly attached to a main stem were used. All leaves which subtended a branching floral or vegetative stem were sampled on any given main stem. After harvest in 1979, lateral leaflets from the regrowth at the late bud stage were measured using a 1-5 scale (MRS) with dimensions devised from a metric rule (1 = 2.0 x 0.5 cm, 2 = 2.5 x 1.0 cm, 3 = 3.0 x 15. cm, 4 = 3.5 x 2.0 cm, and 5 = 4.0 x 2.5 cm). Several fully expanded lateral leaflets from five random plants in each progeny row were scored for each plant. Regrowth score was also measured from each row as for the leaf color study. The initial growth at 100 percent bloom was sampled in the same manner using the metric ruler score in 1980.

Data obtained from the leaf area meter readings and metric ruler scores were analyzed using an analysis of variance on the means. Pearson and Spearman rank correlations were also used to determine how well progeny means correlated across environments and under different rating systems.

Estimation of Heritability

Using a random model, variance components were estimated from the analysis of variance by equating observed mean squares to their expectations and solving for the appropriate variance components.

Estimates of genotypic variance and narrow-sense heritability were obtained from the analyses as follows:

Source	Mean Square	Expectation
Within years:		
Replications	MS_R	$\sigma_E^2 + g\sigma_R^2$
Genotypes	MS_G	$\sigma_E^2 + r\sigma_G^2$
Error	MS_E	σ_E^2
$\sigma_G^2 = (MS_G - MS_E)/r$		
$H_N = 4\sigma_G^2/(4\sigma_G^2 + \sigma_E^2/r)$		
Across years:		
Replications	MS_R	$\sigma_E^2 + gy\sigma_R^2$
Years	MS_Y	$\sigma_E^2 + r\sigma_{GY}^2 + rg\sigma_Y^2$
Genotypes	MS_G	$\sigma_E^2 + r\sigma_{GY}^2 + ry\sigma_G^2$
Genotypes x Years	MS_{GY}	$\sigma_E^2 + r\sigma_{GY}^2$
Error	MS_E	σ_E^2
$\sigma_G^2 = (MS_G - MS_{GY})/ry$		
$H_N = 4\sigma_G^2/(4\sigma_G^2 + 4\sigma_{GY}^2/r + \sigma_E^2/ry)$		

Predicted gains were estimated using the genetic gain formula (9):

$$\hat{G} = H_N(\sigma_A)(K)$$

where: $\hat{G}$ = predicted gain from selection;

H_N = the heritability coefficient;

σ_A = the phenotypic standard deviation of the progenies; and

K = the selection differential.

The selection differential was 2.06 based on a 5 percent selection intensity (9). Gains were added to or subtracted from phenotypic means to estimate predicted means ($\hat{\bar{X}}$).

Results and Discussion

Leaf Color

Significant differences were detected for visual color rating among progenies in 1979. The mean of the 30 dark-leaved progenies tested was 3.36, significantly higher than the mean of the five check cultivars at 3.12. The mean of the pale-leaved progenies (2.80) was significantly lower than the mean of the five check cultivars. Frequency histograms for visual color rating in 1979 are plotted in Figure VI-1. Significant differences were also detected among the individual progenies for visual color rating and for regrowth score (Table VI-1). Five progenies, DK1-5, DK1-24, DK1-32, DK1-42, and DK1-46, had significantly greater visual color ratings than Thor, and two progenies, PA1-15 and PA1-23,

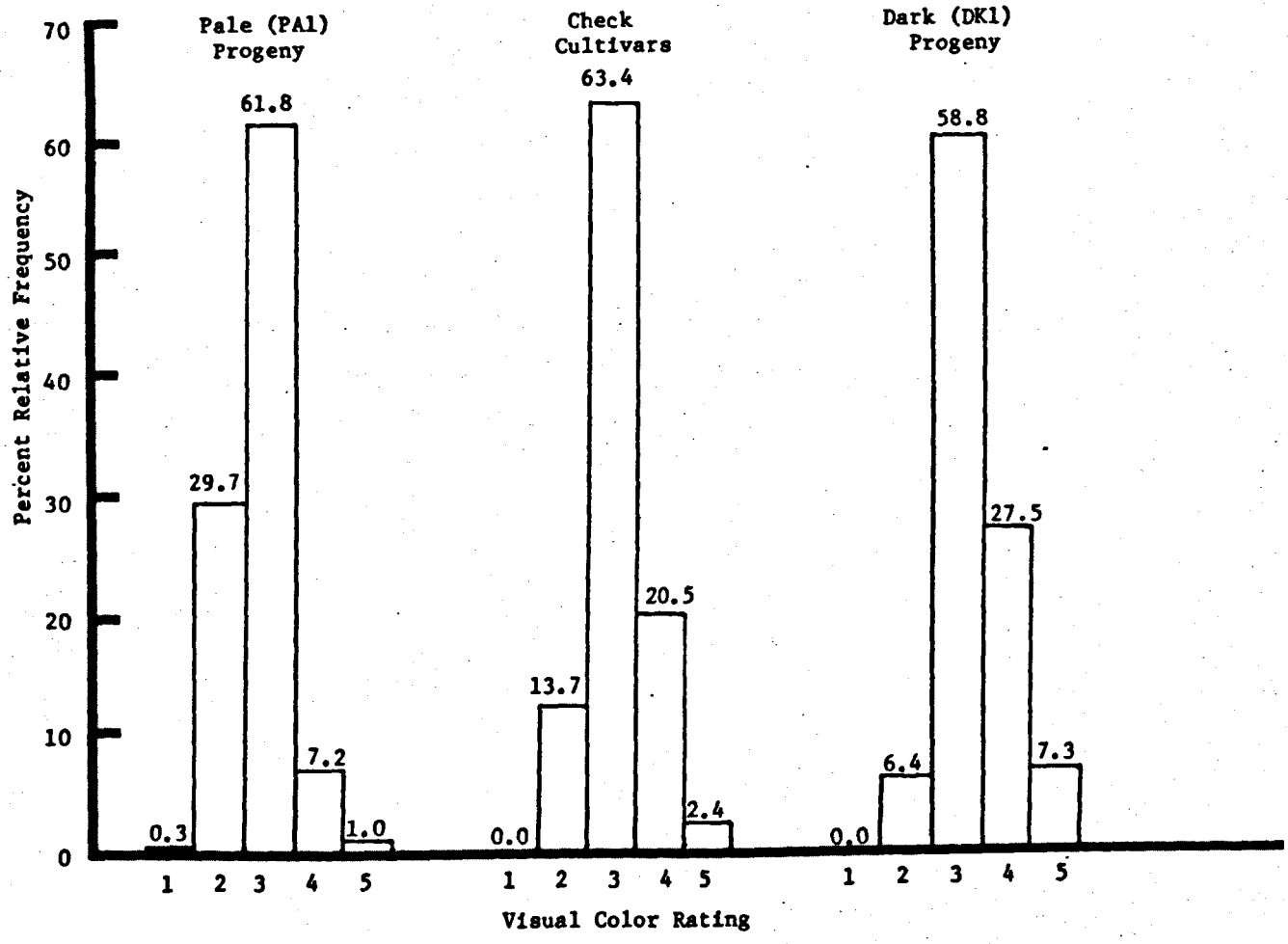

Figure VI-1. Relative frequency distributions for visual color rating (1 = pale yellow-green; 5 = dark blue-green) for individual plants from the PA1 progenies, DK1 progenies, and five check cultivars grown in a replicated progeny trial at Bozeman, Montana in 1979.

Table VI-1. Mean visual color ratings (VCR), Munsell scores (MS), and regrowth scores (RS) for the progeny of 30 dark-leaved (DK1) clones and 21 pale-leaved (PA1) clones, and for five commercial cultivars from a replicated progeny trial at Bozeman, Montana in 1979.

Clone	No. of plants eval.	VCR	No. of plants eval.	MS	RS	Clone	No. of plants eval.	VCR	No. of plants eval.	MS	RS
PA1-23	62	2.41	20	1.65	2.75	Ladak 65	83	2.97	20	2.10	2.50
PA1-15	78	2.57	20	1.15	3.00	DK1-12	72	2.98	20	1.90	3.50
PA1-17	64	2.67	20	1.30	3.75	DK1-19	63	3.02	20	1.85	4.25
PA1- 3	60	2.68	20	1.35	3.25	Anchor	89	3.03	20	2.00	3.75
PA1-10	62	2.68	20	1.45	3.00	PA1- 7	55	3.05	20	2.15	3.75
PA1- 8	72	2.69	20	1.60	3.75	DK1-21	44	3.06	20	2.10	2.00
PA1-12	76	2.70	20	1.55	3.50	DK1- 1	65	3.08	20	2.10	3.00
PA1-20	66	2.71	20	1.55	3.00	DK1-39	54	3.12	20	2.35	2.75
PA1-28	66	2.77	20	1.80	3.50	DK1-20	61	3.16	20	2.00	3.75
PA1-27	48	2.78	20	1.65	2.75	DK1-28	66	3.19	20	2.05	3.75
PA1-19	65	2.81	20	1.20	4.75	DK1-10	59	3.23	20	2.35	2.50
PA1- 2	57	2.82	20	1.95	2.75	DK1-29	53	3.23	20	2.25	3.50
PA1- 5	57	2.83	20	2.02	3.00	DK1-45	57	3.26	20	2.45	3.00
PA1-25	33	2.86	20	1.70	2.50	DK1-23	45	3.30	20	2.35	3.00
PA1-14	56	2.87	20	1.55	4.25	Thor	84	3.32	20	2.20	3.75
PA1-16	49	2.89	20	1.50	3.25	PA1-13	48	3.33	20	2.20	2.75
PA1-31	43	2.89	20	1.75	3.00	Vernal	71	3.33	20	2.10	3.00
DK1- 3	53	2.89	20	2.00	3.50	DK1-22	64	3.33	20	2.25	2.50
PA1- 4	44	2.90	20	1.90	3.25	DK1-30	65	3.33	20	2.12	3.00
PA1-22	55	2.92	20	1.80	4.00	DK1-25	54	3.35	20	2.40	2.50
Ranger	68	2.96	20	1.60	3.00	DK1-13	52	3.36	20	2.30	2.75
DK1-26	58	2.96	20	1.90	4.00	DK1-33	41	3.38	20	2.10	3.50

(table continued)

Table VI-1. continued.

Clone	No. of plants eval.	VCR	No. of plants eval.	MS	RS	Clone	No. of plants eval.	VCR	No. of plants eval.	MS	RS
DK1-50	42	3.39	20	2.40	2.50	DK1-32	64	3.71	20	2.30	2.75
DK1-40	54	3.40	20	2.00	3.00	DK1- 5	59	3.72	20	2.30	3.00
DK1- 2	59	3.49	20	2.35	3.00	DK1-42	54	3.79	20	2.55	2.75
DK1-48	58	3.49	20	2.20	3.25	DK1-46	52	4.16	20	2.79	2.75
DK1- 9	40	3.57	20	2.30	2.75						
DK1- 7	34	3.61	20	2.64	2.50	LSD (0.05)		0.31			1.06
DK1-11	45	3.61	20	2.20	3.50	CV		7.1%			24.3%
DK1-24	23	3.67	20	2.32	2.50						

had significantly lower visual color ratings than Ranger, the highest and lowest ranking cultivars, respectively.

Correlations among means for visual color rating and regrowth score, and Munsell score and regrowth score were -0.16 and -0.29*, respectively. Thus a relationship may exist between increased regrowth and pale leaf color. The correlation between visual color rating and Munsell score was 0.85**, and the Spearman rank correlation was 0.87**. Thus a visual color and Munsell score rating systems give similar results and color scores do not appear to vary from first growth to first harvest regrowth.

Significant differences were also detected for visual color rating among progenies in 1980. The mean of the 30 dark-leaved progenies tested was 3.67, significantly higher than the mean of the five check cultivars at 3.23. The mean of the pale-leaved progenies (2.50) was significantly lower than the mean of the five check cultivars. Frequency histograms for visual color rating in 1980 are shown in Figure VI-2. Significant differences were also detected among the individual progenies for visual color rating in 1980 (Table VI-2). Eight progenies, DK1-1, DK1-3, DK1-5, DK1-9, DK1-10, DK1-13, DK1-42, and DK1-48, had significantly higher visual color ratings than Thor and 12 progenies, PA1-3, PA1-4, PA1-10, PA1-14, PA1-15, PA1-16, PA1-17, PA1-19, PA1-20, PA1-23, PA1-27, and PA1-31, had significantly lower visual color ratings than Ladak 65, the highest and lowest ranking

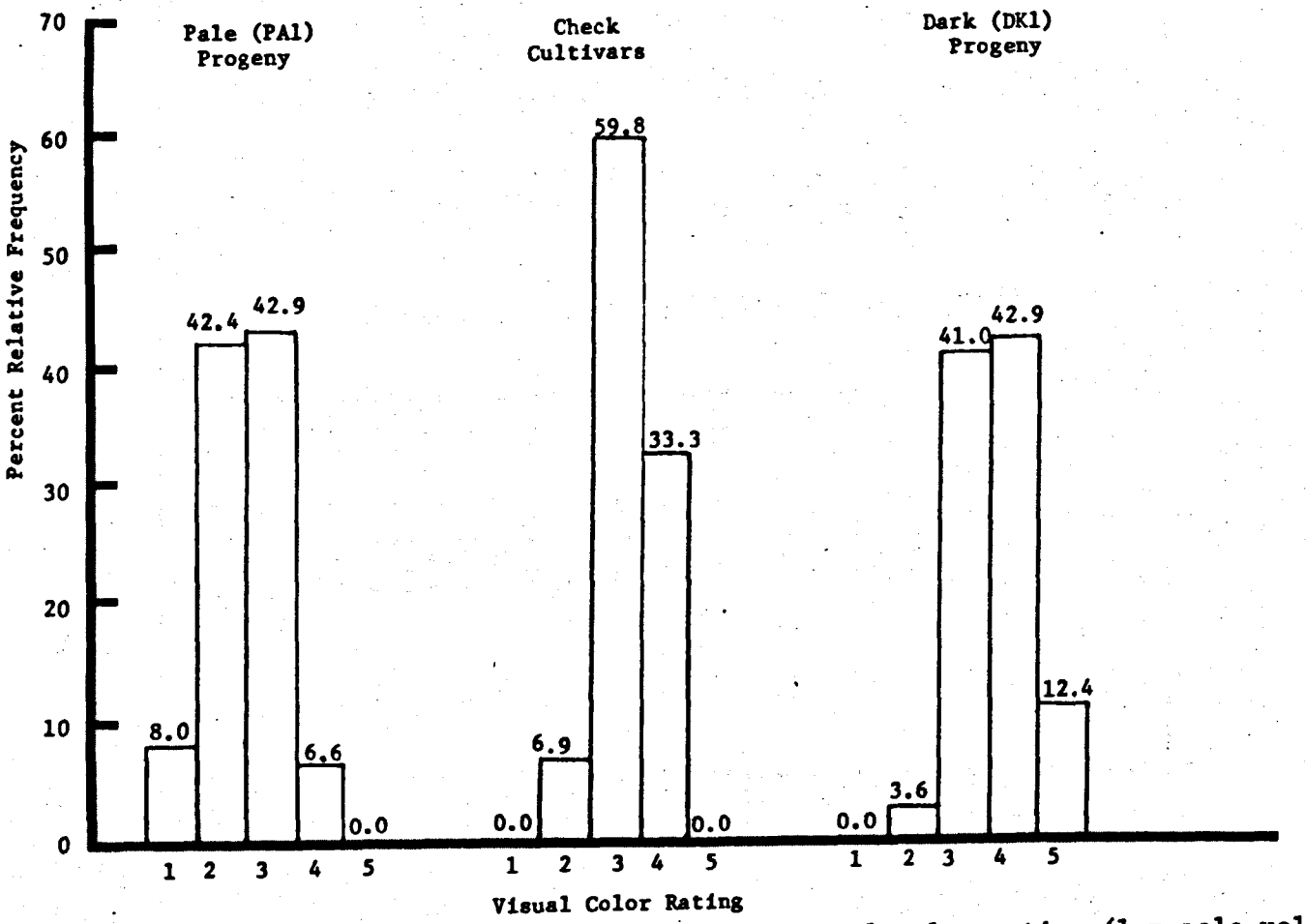

Figure VI-2. Relative frequency distributions for visual color rating (1 = pale yellow-green; 5 = dark blue-green) for individual plants from the PA1 progenies, DK1 progenies, and five check cultivars grown in a replicated progeny trial at Bozeman, Montana in 1980.

Table VI-2. Mean visual color ratings (VCR) and Munsell scores (MS) for the progeny of 30 dark-leaved (DK1) and 21 pale-leaved (PA1) clones, and for five commercial cultivars from a replicated progeny trial at Bozeman, Montana in 1980.

Clone	No. of plants eval.	VCR	No. of plants eval.	MS	Clone	No. of plants eval.	VCR	No. of plants eval.	MS
PA1- 3	20	1.80	20	1.50	DK1-30	20	3.40	20	2.10
PA1-10	20	1.85	20	1.40	DK1-33	20	3.40	20	2.10
PA1-19	20	2.05	20	1.50	Thor	20	3.45	20	2.35
PA1-23	20	2.20	20	1.60	DK1-39	20	3.45	20	2.10
PA1-31	20	2.20	20	1.70	DK1-40	20	3.45	20	2.30
PA1-15	20	2.25	20	1.60	DK1-20	20	3.50	20	2.20
PA1-14	20	2.35	20	1.70	DK1-22	20	3.50	20	2.25
PA1-17	20	2.35	20	1.70	DK1-50	20	3.50	20	2.25
PA1-20	20	2.40	20	1.65	DK1-21	20	3.60	20	2.20
PA1-27	20	2.40	20	1.75	DK1-32	20	3.60	20	2.30
PA1-16	20	2.47	20	1.80	DK1-46	20	3.60	20	2.20
PA1- 4	20	2.50	20	1.80	DK1-11	20	3.65	20	2.25
PA1-12	20	2.70	20	1.75	DK1-45	20	3.70	20	2.30
PA1-22	20	2.70	20	1.95	DK1-24	20	3.75	20	2.35
PA1-28	20	2.70	20	2.00	DK1-29	20	3.80	20	2.25
PA1- 5	20	2.80	20	1.90	DK1- 7	20	3.85	20	2.40
PA1- 8	20	2.85	20	2.05	DK1-25	20	3.85	20	2.35
PA1-25	20	2.90	20	2.00	DK1-28	20	3.85	20	2.20
PA1- 7	20	2.95	20	2.10	DK1- 5	20	3.90	20	2.30
PA1-13	20	3.00	20	2.15	DK1-10	20	3.90	20	2.30
PA1- 2	20	3.05	20	2.00	DK1-48	20	3.90	20	2.45
Ladak 65	20	3.10	20	2.05	DK1- 3	20	3.95	20	2.40
DK1-12	20	3.10	20	2.05	DK1- 1	20	4.10	20	2.50
Ranger	20	3.15	20	2.00	DK1-42	20	4.10	20	2.40
Vernal	20	3.20	20	2.05	DK1-13	20	4.15	20	2.40
Anchor	20	3.25	20	2.10	DK1- 9	20	4.27	20	2.53
DK1- 2	20	3.25	20	2.10					
DK1-19	20	3.25	20	2.10					
DK1-26	20	3.35	20	2.15	LSD (0.05)		0.43		
DK1-23	20	3.40	20	2.20	CV		9.8%		

cultivars, respectively. The correlation among the means for visual color rating and Munsell score was 0.97** and the Spearman rank correlation was 0.96**. This high correlation again indicates that visual color rating and Munsell score are very similar.

In the combined analysis of visual color rating over years, significant differences were detected among progenies. The 2-year mean of the dark-leaved progenies tested was 3.52, significantly higher than the five check cultivars at 3.18. The mean of the pale-leaved progenies (2.65) was significantly lower than the mean of the five check cultivars. The overall frequency histograms for visual color rating are shown in Figure VI-3. Significant differences were also detected among the individual progenies for visual color rating (Appendix table 6). Nine progenies, DK1-5, DK1-7, DK1-9, DK1-13, DK1-24, DK1-32, DK1-42, DK1-46, and DK1-48, had significantly greater visual color ratings than Thor and 15 progenies, PA1-3, PA1-4, PA1-8, PA1-10, PA1-12, PA1-14, PA1-15, PA1-16, PA1-17, PA1-19, PA1-20, PA1-23, PA1-27, PA1-28, and PA1-31, had significantly lower visual color ratings than Ladak 65, the highest and lowest ranking cultivars, respectively (Table VI-3).

Significant differences were also detected between years and for progenies x years indicating that visual color rating may vary in different environments. Correlations among the color variables were all significant (Table VI-4) showing good repeatability and close agreement between visual color and Munsell score rating systems.

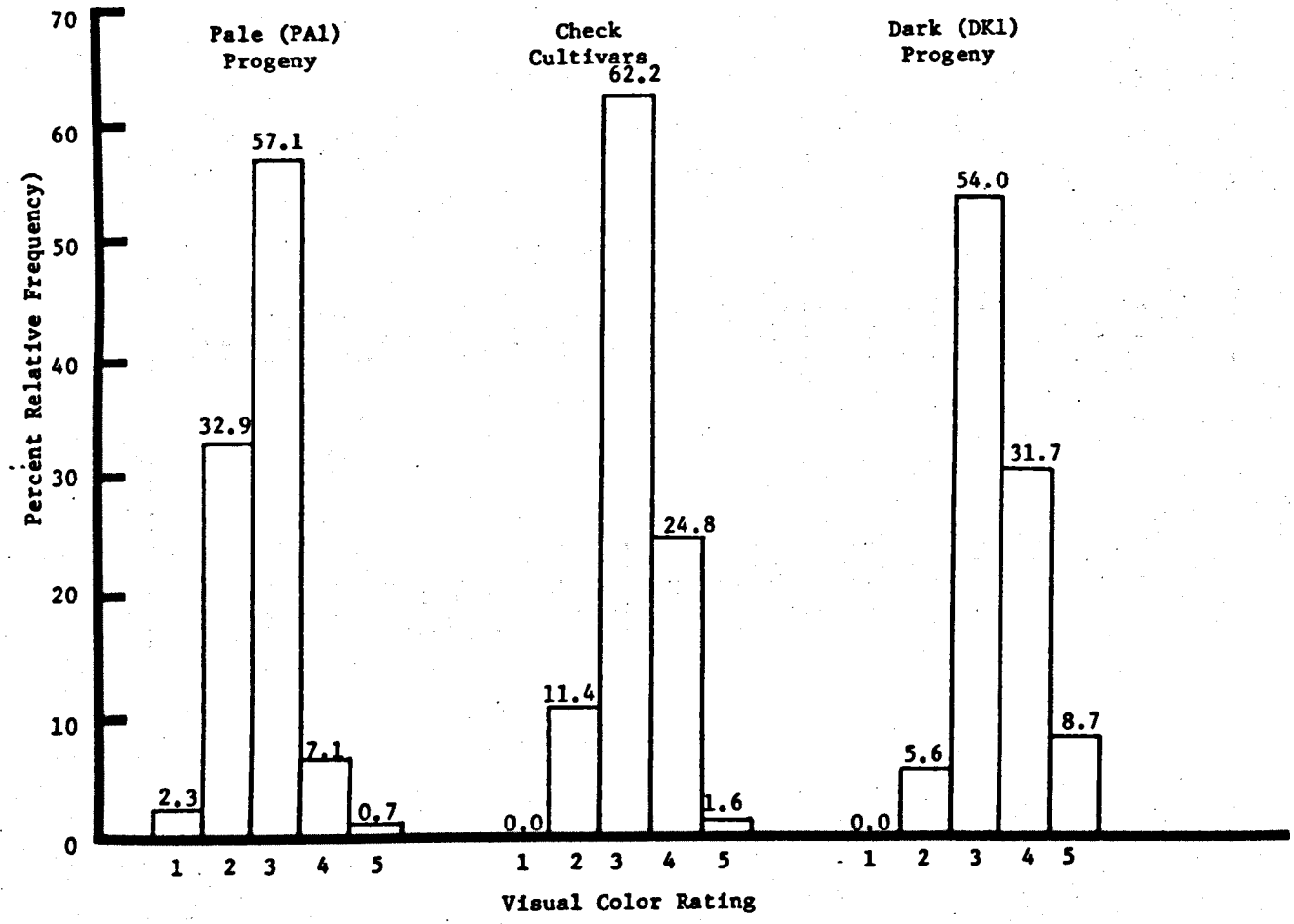

Figure VI-3. Relative frequency distributions for visual color rating (1 = pale yellow-green; 5 = dark blue-green) for individual plants from the PA1 progenies, DK1 progenies, and five check cultivars grown in a replicated progeny trial at Bozeman, Montana combined in 1979 and 1980.

Table VI-3. Mean visual color ratings (VCR) and Munsell scores (MS) for the progeny of 30 dark-leaved (DK1) and 21 pale-leaved (PA1) clones, and for five commercial cultivars from a replicated progeny trial at Bozeman, Montana combined over 1979 and 1980.

Clone	VCR	MS	Clone	VCR	MS
PA1-10	2.26	1.42	DK1-21	3.33	2.15
PA1- 3	2.28	1.42	DK1-23	3.35	2.27
PA1-23	2.31	1.62	DK1- 2	3.37	2.22
PA1-15	2.41	1.37	DK1-30	3.37	2.11
PA1-19	2.43	1.35	Thor	3.38	2.27
PA1-17	2.51	1.50	DK1-33	3.39	2.10
PA1-31	2.54	1.72	DK1-22	3.41	2.25
PA1-20	2.55	1.60	DK1- 3	3.42	2.20
PA1-27	2.59	1.70	DK1-40	3.43	2.15
PA1-14	2.61	1.62	DK1-50	3.44	2.30
PA1-16	2.68	1.65	DK1-45	3.48	2.37
PA1- 4	2.70	1.85	DK1-28	3.52	2.12
PA1-12	2.70	1.65	DK1-29	3.52	2.25
PA1-28	2.73	1.90	DK1-10	3.56	2.32
PA1- 8	2.77	1.82	DK1- 1	3.59	2.30
PA1- 5	2.81	1.96	DK1-25	3.60	2.37
PA1-22	2.81	1.87	DK1-11	3.63	2.22
PA1-25	2.88	1.85	DK1-32	3.66	2.30
PA1- 2	2.93	1.97	DK1-48	3.70	2.32
PA1- 7	3.00	2.12	DK1-24	3.71	2.34
Ladak 65	3.03	2.07	DK1- 7	3.73	2.52
DK1-12	3.04	1.97	DK1-13	3.76	2.35
Ranger	3.05	1.80	DK1- 5	3.81	2.30
Anchor	3.14	2.05	DK1-46	3.88	2.49
DK1-19	3.14	1.97	DK1- 9	3.92	2.42
PA1-13	3.16	2.17	DK1-42	3.95	2.47
DK1-26	3.16	2.02			
Vernal	3.27	2.07	LSD (0.05)	0.25	
DK1-39	3.28	2.22	CV	8.2%	
DK1-20	3.33	2.10			

Table VI-4. Pearson (r) and Spearman (r_s) correlation coefficients between color variables for several field-grown alfalfa progenies at Bozeman, Montana in 1979 and 1980.

Variable pair	r	r_s
VCR(1979) and MS(1979)	0.852**	0.869**
VCR(1979) and VCR(1980)	0.758**	0.796**
VCR(1979) and MS(1980)	0.752**	0.813**
VCR(1979) and MS(1979-80)	0.844**	0.883**
VCR(1980) and MS(1979)	0.830**	0.803**
VCR(1980) and MS(1980)	0.967**	0.960**
VCR(1980) and MS(1979-80)	0.927**	0.914**
MS(1979) and MS(1980)	0.835**	0.811**
MS(1979) and VCR(1979-80)	0.889**	0.870**
MS(1980) and VCR(1979-80)	0.944**	0.946**
MS(1979-80) and VCR(1979-80)	0.952**	0.954**

**Significant at .01 probability level.

Heritabilities for dark and pale leaf color assessed by visual color rating were high (Table VI-5). Predicted genetic gains (Table VI-5) varied from 0.43 to 0.55 for the DK1 progenies and from 0.32 to 0.70 for the PA1 progenies indicating that significant progress should be realized for both dark and pale leaf color following another selection cycle.

Leaf color in alfalfa is easily assessed by visual color rating and is both efficient and repeatable. Expression of the leaf color trait varied little from first growth to regrowth. Spearman rank correlations indicated that the relative order of visual color ratings among the progenies did not change across years.

Selection for either dark or pale leaf color in alfalfa should be successful and this trait could be used in a breeding program to assess drought resistance in alfalfa.

Leaflet Size

Significant differences were detected among progenies for leaflet size (cm^2) using the leaf area meter (LAM) in 1979. The mean of the 30 large-leaved progenies was 1.74 cm^2, not significantly greater than the mean of the five check cultivars at 1.59 cm^2. The mean of the 19 small-leaved progenies (1.23 cm^2) was significantly lower than the mean of the five check cultivars. Relative frequency distributions for each

Table VI-5. Genetic variance components (σ^2_G), heritabilities (H_N), predicted gains ($\hat{G}$), and predicted means ($\hat{\bar{X}}$) for dark and pale leaf color using visual color ratings in 1979, 1980, and combined in 1979 and 1980 estimated from a replicated progeny trial at Bozeman, Montana.

Population	σ^2_G	H_N	$\hat{G}$	$\hat{\bar{X}}$
DK1 progenies (1979)	.010	72.7%	0.43	3.79
PA1 progenies (1979)	.032	93.4%	0.36	2.44
DK1 progenies (1980)	.063	91.3%	0.55	4.22
PA1 progenies (1980)	.113	94.2%	0.70	1.81
DK1 progenies (1979-80)	.056	94.7%	0.45	3.96
PA1 progenies (1979-80)	.026	63.8%	0.32	2.33

group are shown in Figure VI-4. Significant differences were also detected among the individual progenies for leaf area (Table VI-6). Three progenies, LG1-12, LG1-14, and LG1-34 had significantly greater leaf area than Ranger, and eight progenies, SM1-3, SM1-14, SM1-15, SM1-22, SM1-23, SM1-28, SM1-31, and SM1-32, had lower leaf area than Vernal, the cultivars with the largest and smallest leaf area, respectively.

Significant differences were detected among progenies for metric ruler score (MRS) in 1979. The mean of the 30 large-leaved progenies was 3.33, significantly greater than the mean of the five check cultivars at 3.16. The mean of the 19 small-leaved progenies (2.33) was significantly lower than the mean of the five check cultivars. Relative frequency distributions for each group are shown in Figure VI-5. Significant differences were also detected among the individual progenies for metric ruler score and regrowth score (Table VI-6). One progeny, LG1-3, had significantly higher metric ruler score than Thor, and seven progenies, SM1-13, SM1-14, SM1-15, SM1-22, SM1-28, SM1-31, and SM1-32, had lower metric ruler scores than Ladak 65, the cultivars with the highest and lowest metric ruler scores, respectively.

Correlations among the means for leaf area and regrowth score, and metric ruler score and regrowth score, in 1979 were 0.35* and 0.73*, respectively, indicating that plants with larger leaflets have more vigorous regrowth. The correlation between leaf area and metric ruler score was 0.77**, and the Spearman rank correlation was 0.78**.

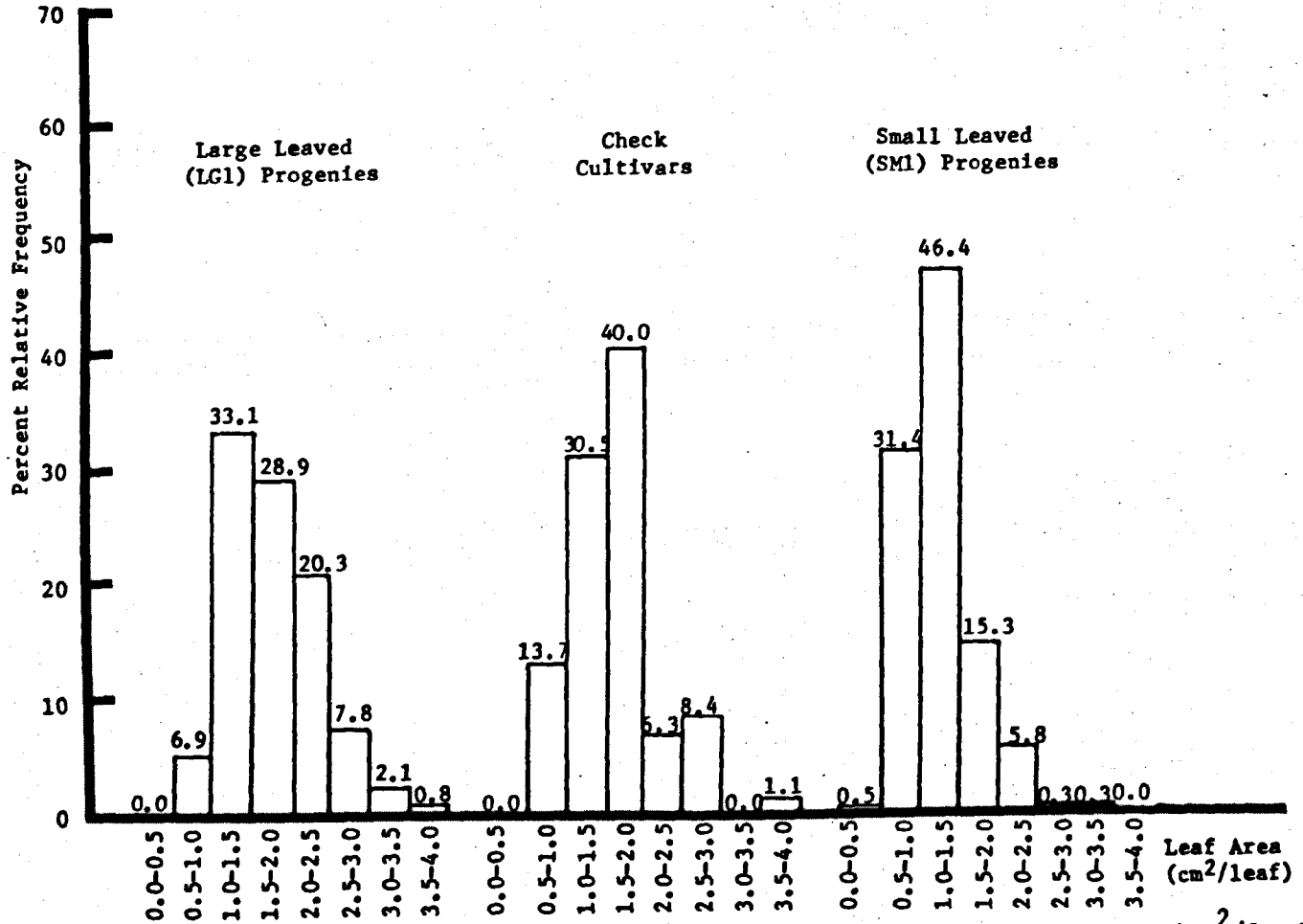

Figure VI-4. Relative frequency distributions for leaf area meter readings (cm^2/leaf) for individual plants from the LG1 progenies, SM1 progenies, and five check cultivars grown in a replicated progeny trial at Bozeman, Montana in 1979.

Table VI-6. Leaf area meter (LAM) readings, metric ruler scores (MRS), and regrowth scores (RS) for cycle one large- (LG1) and small-leaved (SM1) progenies, and five check cultivars in 1979.

Progeny	No. of plants	LAM	No. of plants	MRS	RS
SM1-28	20	0.84	20	1.25	1.75
SM1-14	5	1.08	3	1.67	2.00
SM1-22	10	0.96	7	1.67	2.00
SM1-15	15	0.91	20	1.90	2.50
SM1-31	20	1.08	18	1.90	2.00
SM1-32	20	0.97	11	1.92	1.50
SM1-13	20	1.39	20	2.15	2.25
SM1-23	20	1.05	20	2.30	2.75
SM1-21	10	1.30	10	2.33	2.00
SM1-39	20	1.42	20	2.45	2.50
SM1-33	20	1.29	20	2.50	3.00
LG1-17	10	1.30	7	2.50	2.00
SM1- 4	15	1.22	20	2.60	2.75
SM1- 6	20	1.52	20	2.70	2.75
SM1- 8	20	1.16	20	2.70	3.00
LG1-10	20	1.29	20	2.70	2.50
SM1- 7	20	1.42	20	2.75	2.75
SM1-12	10	1.60	5	2.80	2.50
Ladak 65	20	1.49	20	2.80	2.50
SM1- 3	20	1.11	20	2.85	3.00
SM1- 5	20	1.20	20	2.90	3.00
SM1-35	20	1.42	20	2.45	2.50
Vernal	20	1.48	20	2.90	3.00
LG1-15	20	1.70	20	2.95	3.00
LG1-50	20	1.66	20	2.95	3.00
LG1- 6	20	1.65	20	3.00	2.75
LG1-24	20	1.29	20	3.00	3.00
LG1- 9	20	1.72	14	3.05	2.50
LG1-29	20	1.74	20	3.05	2.75
LG1-11	15	1.22	18	3.07	2.75
LG1-22	20	1.71	20	3.10	3.25
Anchor	20	1.66	20	3.20	3.75
LG1- 8	20	1.65	20	3.20	3.00
LG1-39	20	1.90	20	3.24	3.25

(table continued)

Table VI-6. continued.

Progeny	No. of plants	LAM	No. of plants	MRS	RS
LG1-14	20	2.16	20	3.25	2.75
LG1-44	20	1.92	20	3.25	2.75
LG1-30	20	1.75	19	3.34	2.75
Ranger	20	1.76	20	3.40	3.75
LG1-47	20	1.94	20	3.40	3.75
LG1- 1	20	1.32	20	3.45	3.75
LG1- 5	20	1.44	20	3.45	3.25
LG1-32	20	1.79	20	3.45	3.00
Thor	20	1.55	20	3.50	3.75
LG1-12	20	2.37	20	3.50	3.25
LG1-19	20	1.78	20	3.50	3.50
LG1-37	20	1.62	20	3.50	3.50
LG1-13	20	1.57	19	3.55	3.50
LG1-40	20	1.97	20	3.60	3.50
LG1-45	20	1.82	20	3.65	3.25
LG1-23	20	2.07	20	3.70	4.00
LG1-18	15	1.75	20	3.75	3.75
LG1-21	20	1.81	20	3.80	4.00
LG1-34	20	2.14	18	3.95	3.50
LG1- 3	20	1.93	20	4.10	4.50
LSD (0.05)		0.36		0.51	0.66
CV		16.7%		12.4%	16.0%

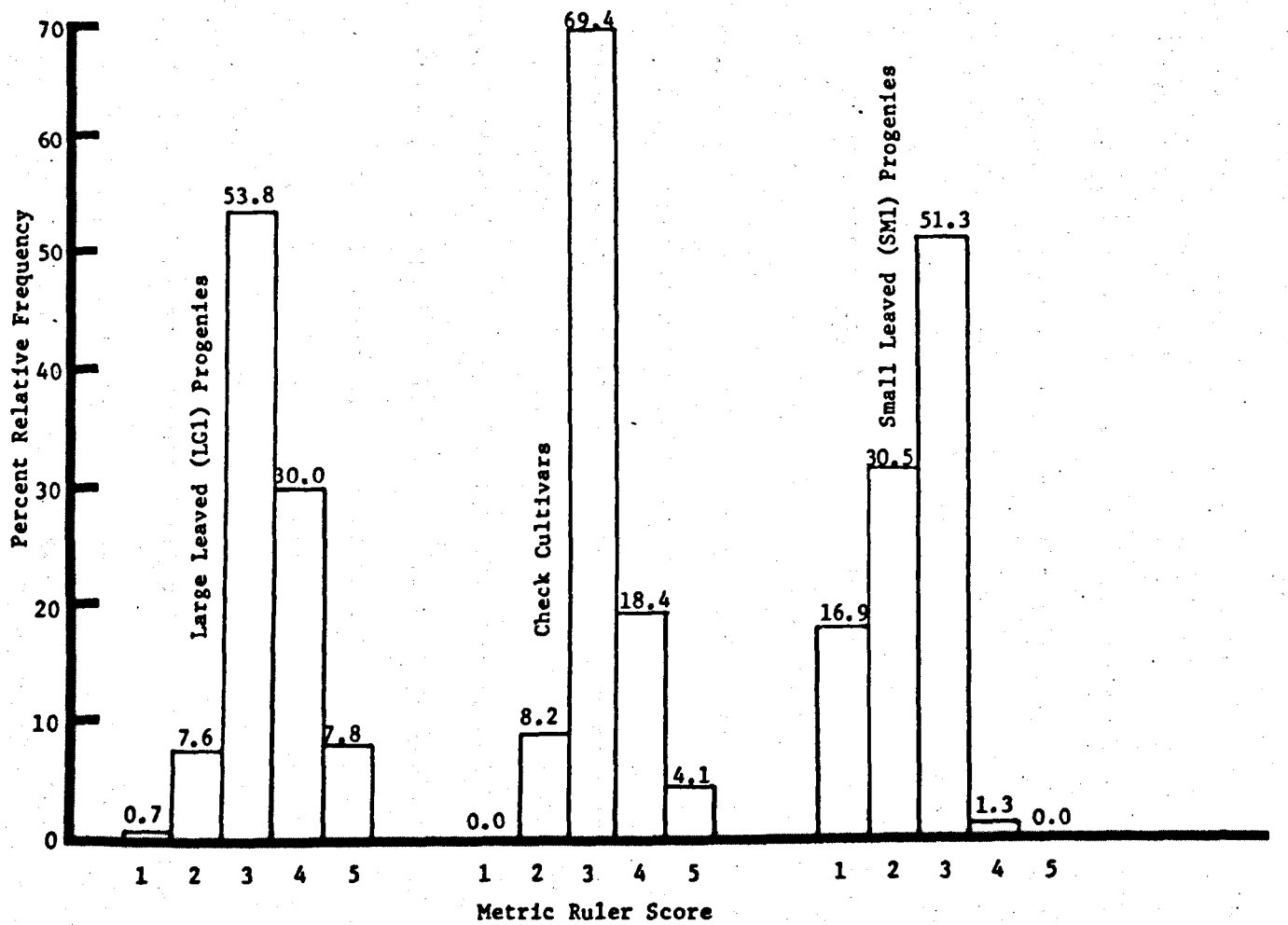

Figure VI-5. Relative frequency distributions for metric ruler score (1 = 2.0 x 0.5 cm; 5 = 4.0 x 2.5 cm) for individual plants from the LG1 progenies, SM1 progenies, and five check cultivars grown in a replicated progeny trial at Bozeman, Montana in 1979.

Thus, leaf area and metric ruler score give similar results and leaf area for any given progeny does not vary appreciably from first growth to first harvest regrowth.

Significant differences were also detected for metric ruler score among progenies in 1980. The mean of the 30 large-leaved progenies tested was 3.66, significantly higher than the mean of the five check cultivars at 3.31. The mean of the 19 small-leaved progenies (2.61) was significantly lower than the mean of the five check cultivars. Frequency histograms for metric ruler score in 1980 are shown in Figure VI-6. Significant differences were also detected among the individual progenies for metric ruler score in 1980 (Table VI-7). Five progenies, LG1-3, LG1-6, LG1-12, LG1-18, and LG1-34, had significantly greater metric ruler scores than Ranger, and nine progenies, SM1-5, SM1-12, SM1-15, SM1-21, SM1-22, SM1-23, SM1-28, SM1-31, and SM1-32, had lower metric ruler scores than Vernal, the cultivars with the highest and lowest metric ruler scores, respectively.

In the combined analysis of metric ruler score over years, significant differences were detected among progenies. The 2-year mean of the large-leaved progenies tested was 3.50, significantly higher than the mean of the check cultivars at 3.23. The mean of the check cultivars was significantly higher than the mean of the small-leaved progenies (2.47). Frequency histograms for metric ruler score combined for 1979-80 are plotted in Figure VI-7. Significant differences were

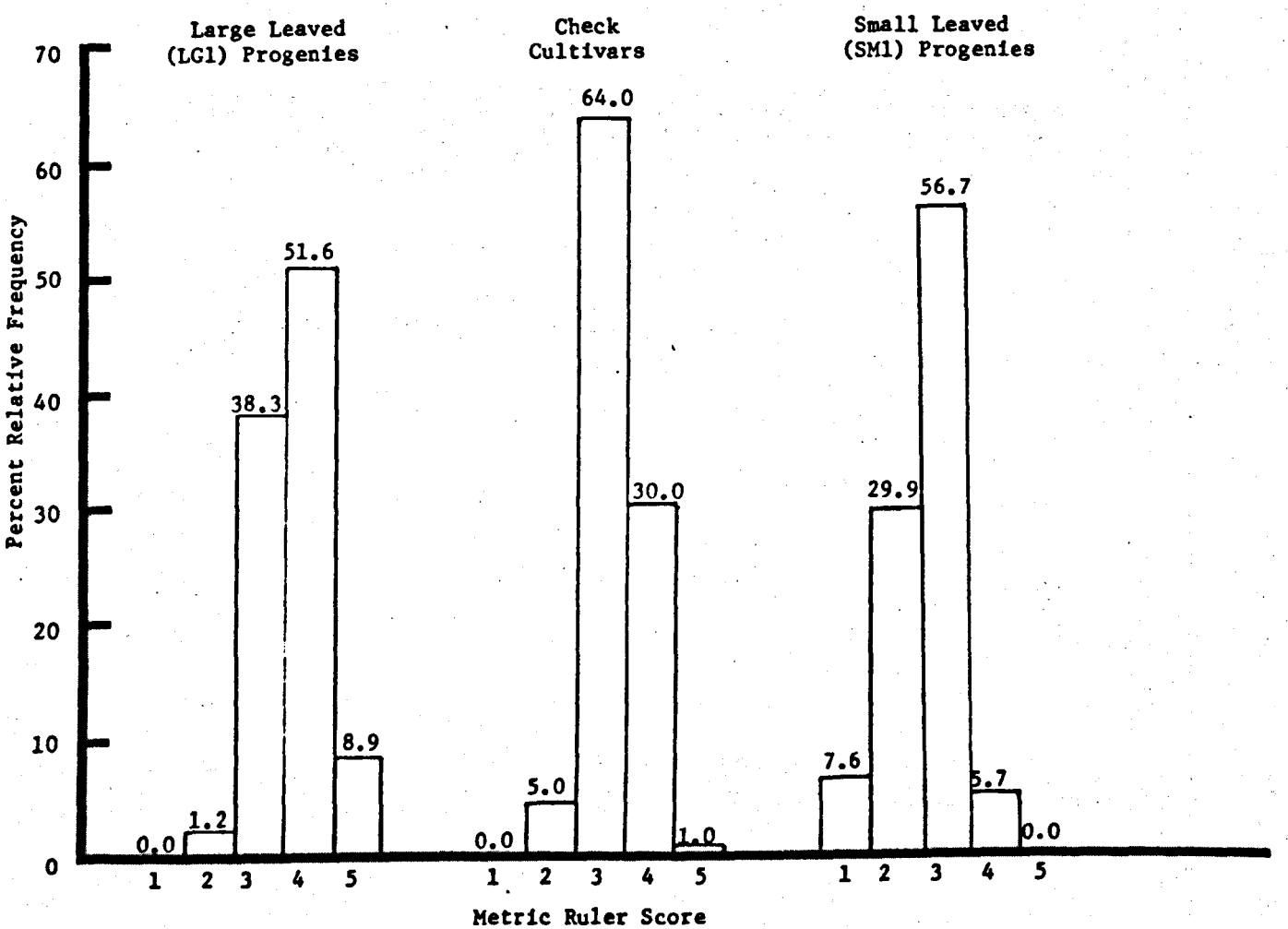

Figure VI-6. Relative frequency distributions for metric ruler score (1 = 2.0 x 0.5 cm; 5 = 4.0 x 2.5 cm) for individual plants from the LG1 progenies, SM1 progenies, and five check cultivars grown in a replicated progeny trial at Bozeman, Montana in 1980.

Table VI-7. Metric ruler scores (MRS) for cycle one large- (LG1) and small-leaved (SM1) progenies, and five check cultivars in 1980 and combined 1979-80.

Progeny	No. of plants	MRS	
		1980	1979-80
SM1-28	20	1.60	1.42
SM1-31	20	2.15	2.02
SM1-32	10	2.20	2.06
SM1-22	10	2.60	2.19
SM1-15	20	2.55	2.22
SM1-23	20	2.30	2.30
SM1-14	5	3.00	2.33
SM1-21	10	2.40	2.37
SM1-13	20	2.70	2.42
SN1-33	20	2.80	2.65
SM1-39	20	2.85	2.65
SM1-12	10	2.60	2.70
SM1- 8	20	2.75	2.72
SM1- 5	20	2.60	2.75
SM1- 7	20	2.75	2.75
SM1- 6	20	2.85	2.77
SM1- 3	20	2.80	2.82
SM1-35	20	2.80	2.85
LG1-10	20	3.00	2.85
LG1-17	5	3.20	2.85
Vernal	20	3.00	2.95
SM1- 4	20	3.10	2.97
Ladak 65	20	3.30	3.05
LG1-24	20	3.35	3.17
LG1- 8	20	3.25	3.22
LG1-50	20	3.55	3.25
LG1- 9	15	3.47	3.26
LG1-11	20	3.50	3.28
Anchor	20	3.45	3.32
LG1-44	20	3.45	3.35
Thor	20	3.30	3.40
LG1-15	20	3.85	3.40
LG1-22	20	3.70	3.40
LG1-30	20	3.50	3.42
LG1-39	20	3.65	3.44
Ranger	20	3.50	3.45

(table continued)

Table VI-7. continued.

Progeny	No. of plants	MRS	
		1980	1979-80
LG1-29	20	3.85	3.45
LG1- 6	20	3.95	3.47
LG1-19	20	3.45	3.47
LG1-47	20	3.55	3.47
LG1-40	20	3.45	3.52
LG1-14	20	3.85	3.55
LG1-13	20	3.65	3.60
LG1- 1	20	3.80	3.62
LG1- 5	20	3.80	3.62
LG1-23	20	3.55	3.62
LG1-32	20	3.80	3.62
LG1-12	20	3.90	3.70
LG1-37	20	3.85	3.70
LG1-45	20	3.85	3.75
LG1-21	20	3.80	3.80
LG1-18	20	4.05	3.90
LG1-34	20	4.10	4.02
LG1- 3	20	4.10	4.10
LSD (0.05)		0.39	0.32
CV		8.7%	10.6%

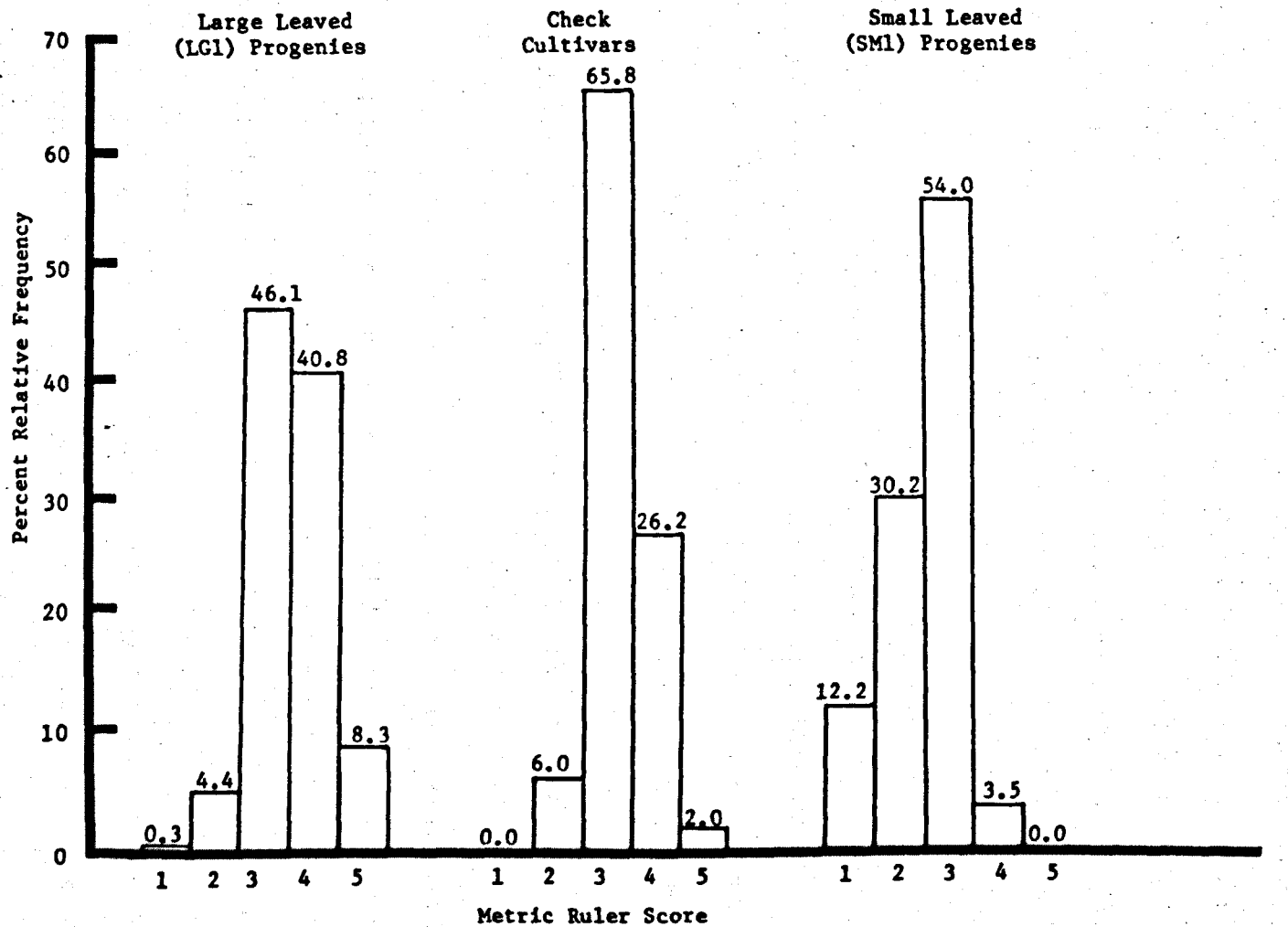

Figure VI-7. Relative frequency distributions for metric ruler score (1 = 2.0 x 0.5 cm; 5 = 4.0 x 2.5 cm) for individual plants from the LG1 progenies, SM1 progenies, and five check cultivars grown in a replicated progeny trial at Bozeman, Montana combined in 1979 and 1980.

also detected among the individual progenies for metric ruler score (Table VI-7). Four progenies, LG1-3, LG1-18, LG1-21, and LG1-34, had significantly higher metric ruler scores than Ranger, and nine progenies, SM1-13, SM1-14, SM1-15, SM1-21, SM1-22, SM1-23, SM1-28, SM1-31, and SM1-32, had significantly lower metric ruler scores than Vernal, the highest and lowest ranking cultivars, respectively (Table VI-7).

Significant differences were also detected for blocks, years, and progenies x years (Appendix table 7) indicating that metric ruler scores vary in different environments. Correlations among the leaflet size variables were all significant (Table VI-8) showing good repeatability and close agreement between leaf area meter readings and metric ruler scores.

Heritabilities for large and small leaflet size assessed by the leaf area meter and metric ruler score were high (Table VI-9). Selection for leaflet size through use of the leaf area meter should be very effective for both large and small leaflets. Using metric ruler score combined over years, the large leaf trait and small leaf trait appeared about equally heritable at 84.3% and 85.2%, respectively. Predicted genetic gains (Table VI-9) varied from 0.50 to 0.66 for the LG1 progenies and from 0.68 to 0.90 for the SM1 progenies indicating that significant progress should be realized for both large and small leaflet size following another selection cycle.

Table VI-8. Pearson (r) and Spearman (r_s) rank correlation coefficients between leaflet size variables for several progenies at Bozeman, Montana in 1979 and 1980.

Variable pair	r	r_s
LAM(1979) and MRS(1979)	0.770**	0.785**
LAM(1979) and MRS(1980)	0.759**	0.756**
LAM(1979) and MRS(1979-80)	0.795**	0.798**
MRS(1979) and MRS(1980)	0.850**	0.822**

**Significant at .01 probability level.

Table VI-9. Genetic variance components (σ^2_G), heritabilities (H_N), predicted gains (G), and predicted means ($\hat{X}$) for large and small leaflet size using leaf area meter readings in 1979 and metric ruler scores in 1979, 1980, and combined in 1979 and 1980 estimated from a replicated progeny trial at Bozeman, Montana.

Population	σ^2_G	H_N	$\hat{G}$	$\hat{X}$
Leaf area meter reading				
LG1 progenies (1979)	.103	98.3%	0.56	2.32
SM1 progenies (1979)	.031	91.9%	0.38	0.86
Metric ruler score				
LG1 progenies (1979)	.079	88.8%	0.66	3.99
SM1 progenies (1979)	.112	79.4%	0.90	1.43
LG1 progenies (1980)	.079	94.6%	0.52	4.18
SM1 progenies (1980)	.109	93.6%	0.72	1.89
LG1 progenies (1979-80)	.054	84.3%	0.50	4.00
SM1 progenies (1979-80)	.104	85.2%	0.68	1.79

Leaflet size in alfalfa can be assessed using a leaf area meter or by metric ruler score as both methods are repeatable. Evaluation by metric ruler score is more efficient than using the leaf area meter since it is easier and faster. The leaflet size trait varied little from first growth to regrowth. Spearman rank correlations indicated that the relative order of metric ruler scores among progenies did not change across years.

Selection for either large or small leaflet size in alfalfa should be successful and this trait could be used in a breeding program to assess drought resistance in alfalfa.

CHAPTER VII

ALFALFA SEED GERMINATION IN ANTIBIOTIC AGAR CONTAINING NaCl

Drought resistance and salt tolerance are related (167, 169, 174). The development of cultivars able to germinate under high salt stress would not only be useful in the reclamation of saline soils but might enable improved germination in dry soils. A major problem has been the development of uniform, repeatable selection methods. The objectives of this study were to determine if saline antibiotic agar could be successfully used as a saline medium to rapidly screen large numbers of seeds for ability to germinate at relatively high NaCl levels, and if progress through selection could be made for this trait.

Materials and Methods

Alfalfa seeds from 15 cultivars were mechanically scarified using a Forsberg scarifier containing sandpaper for 5 seconds and divided into 50 lots of 50 seeds each.

Noble agar (Difco) was added (11 g/ℓ) to twice-distilled water. Sodium chloride was added to each of 10 agar solutions at 0.00, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, and 3.00 percent (w/v). Each agar solution was loosely stoppered in a flask with cotton and covered with aluminum foil. The flasks were autoclaved for 20 minutes at 125 C and 1.7 kg/cm^2. After 1 hour, the flasks containing the salt and agar, now in solution, were removed and placed in a 50 C water bath to cool. Upon attaining thermal equilibrium, electrical conductivity measurements

were recorded for each liquid agar solution using a Barnstad Wheatstone Bridge with a glass probe. Antiobiotics were then added to each flask in the following concentrations (a.i. in ppm): 2 ppm Oxytetracycline hydrochloride (Sigma Chemical Co.), 5 ppm Streptomycin sulfate (Sigma Chemical Co.), 10 ppm Benomyl (50% a.i.; E. I. duPont de Nemours and Co.), and 60 ppm Captan (50% a.i.; WP, Chevron Chemical Co.). These rates provided adequate control of pathogens in preliminary tests. To further reduce contamination, the seeds were treated with Captan (2.3 g a.i./kg alfalfa seed) to avoid the need for surface sterilization which could alter germination.

Agar (20 ml per plate) was poured into disposable Petri plates (15 x 100 mm) using an automatic pipetter (Cornwall). The agar had been cooled to 50 C to avoid excessive evaporation and condensation in the plates. When the agar gelled, the electrical conductivity of the plates was measured using the previous system. Alfalfa seeds were poured onto the remaining agar plates under a hood to further reduce contamination. Each plate contained 50 seeds and each treatment combination was replicated five times. The plates were quickly covered, placed in sealed plastic bags and put in a 20 C dark germination chamber. The Petric plates were inverted to minimize condensation on the covers. They were removed from the chamber for seedling counts at 5, 10, 15, and 20 days.

Arcsine square root transformations of percent germination were used in the analysis of variance to stabilize the variances (255). Response surface analyses were conducted for each cultivar.

Progress from selection for the ability to germinate on antibiotic agar containing NaCl was assessed using the cultivar Ladak 65. Ten thousand seeds were tested at the 1.75% level of NaCl and 291 plants that were able to germinate to a radicle length of at least 1 cm were saved (Fig. VII-1). These were transferred to wet blotters without NaCl until they reached the unifoliate leaf stage. They were then transplanted into conetainers and later sent to Reno, Nevada for isolated intercrossing in bee cages.

The seed harvested from 250 of these clones was bulked and returned to Bozeman for testing. This seed, designated as Agar (C-1), along with parental Ladak 65, Thor, Salton, and three other germplasms was tested at 0.00, 0.50, 1.00, 1.50, 1.75, 2.00, and 2.50 percent NaCl (w/v) in agar using identical procedures as were used in the cultivar survey. The three germplasms tested were: Blotter (C-1), cycle one selections made from Ladak 65 for ability to germinate on blotters in germination boxes containing relatively high NaCl levels; 994, seed obtained from Dr. J. B. Moutray, NAPB, Ames, Iowa, which is purported to possess mature plant salt tolerance; and seed obtained from Dr. A. K. Dobrenz, which was harvested from plants surviving on high-pH, dryland soils in Arizona. Similar statistical techniques were again used.

Results and Discussion

Fifteen alfalfa cultivars were germinated in antibiotic agar containing 10 levels of NaCl. Germination counts were made on each of five replications for each treatment combination at 5, 10, 15, and 20 days. Germination percentages were expressed as percent of the no-salt check for each cultivar to account for differences in germination (Table VII-1).

Significant differences were detected for cultivars, days, salt levels, cultivars x salt levels, and days x salt levels (Table VII-2). Good differentiation among cultivars was observed at 1.25, 1.50, and 1.75% NaCl (w/v) for ability to germinate (Table VII-1). Anchor, Baker, and Salton appeared consistently superior and Iroquois, Olympic, and Ranger had the lowest relative percent germination. This technique enables differentiation among genotypes for ability to germinate at relatively high salt levels.

Germination in NaCl increased over time and decreased with increasing salt levels (Table VII-3). The greater the salt concentration, the greater was the influence of time on germination. At 1.75% NaCl more than a tenfold increase in germination resulted from 5 to 20 days, whereas only a 22% increase resulted at the 1.00% level. Very little change occurred over time at either 0.00% or 0.50% NaCl. This phenomenon accounts for the significant days x salt levels interaction.

Table VII-1. Mean adjusted 1/ percent germination by level of NaCl in agar for 15 alfalfa cultivars using sample germination counts. Mean of 5, 10, 15, and 20 days for five replicates.

	Percent NaCl (w/v)							
Cultivar	0.00	0.50	0.75	1.00	1.25	1.50	1.75	2.00
Thor	100	91	90	89	78	36	4	1
Ladak 65	100	98	97	92	68	20	3	1
Vangard	100	85	85	81	68	21	3	0
Vernal	100	100	99	97	80	44	7	0
Olympic	100	91	74	71	55	22	6	0
Apollo	100	95	95	83	74	22	3	0
Drylander	100	100	93	87	75	26	8	1
Ranger	100	94	84	84	58	25	2	0
Grimm	100	98	97	92	85	30	6	0
Anchor	100	96	91	91	85	49	13	1
Iroquois	100	93	90	90	49	22	2	0
Honeoye	100	98	91	91	67	27	2	0
Riley	100	94	91	80	65	25	3	1
Baker	100	94	94	91	91	46	9	1
Salton	100	96	94	88	88	45	5	1
Mean	100	95	91	87	72	31	5	1

1/ Germination expressed as a percent of the 0.00 NaCl check within each cultivar.

Table VII-2. Analysis of variance of arcsine square root transformations of percent germination at 5, 10, 15, and 20 days for 15 alfalfa cultivars at seven NaCl levels in agar.

Source	Degrees of freedom	Mean squares
Cultivars (C)	14	0.693**
Days (D)	3	2.741**
Salt levels (S)	6	92.603**
C x D	42	0.009
C x S	84	0.115**
D x S	18	0.145**
C x D x S	252	0.008
Error	1680	0.010
Total	2099	

**Significant at .01 level.

Table VII-3. Mean change in percent germination for 15 alfalfa cultivars over time at various NaCl levels in agar.

	Percent NaCl				
Time (days)	0.00	0.50	1.00	1.50	1.75
5	83.7	78.5	65.1	14.9	0.6
10	85.9	80.2	75.3	28.0	3.8
15	85.9	82.8	78.4	30.8	5.8
20	85.9	82.8	79.6	33.4	7.3
%ΔG 1/	2.6	5.3	22.3	124.2	1116.7

1/ $\frac{\text{Germ. at 20 days - Germ. at 5 days}}{\text{Germ. at 5 days}} \times 100.$

Electrical conductivity tests revealed that increasing NaCl levels resulted in greater conductivities (Table VII-4). The small standard errors indicate the uniformity of agar as a test medium. The point where germination ceased in most cultivars was at 11-12 atmospheres or above 2.00% NaCl. The optimum level of NaCl to obtain a 5% selection intensity for most cultivars was 1.75% (9-10 atmospheres). This level was used for selection in this study.

Response surface analyses were conducted for each cultivar and the relationship between percent germination and salt level for any cultivar, when the curves were forced through an intercept of 100% germination, appears to be an excellent quadratic fit. P-values ranged from 0.002 to 0.015 and quadratic r^2 values ranged from 0.961 to 0.992. The estimated inhibitory concentration of NaCl which prevents 50% of the seeds from germinating (IC50) has been estimated from the quadratic curves for each cultivar (Table VII-5). Formulae used to calculate the IC50 and an approximation of its associated standard error are:

$$IC50 = -b_1 - [b_1^2 - 4b_2(50)]^{\frac{1}{2}}$$

$$S.E.(IC50) = S.E.(\hat{y}/IC50)[b_1 + 2(b_2)IC50]^{-1}$$

Germination of Anchor, Baker, and Salton were least affected by the NaCl, whereas Iroquois, Olympic, Ranger, and Vangard were most affected.

Table VII-4. Electrical conductivities and standard errors (mmhos/cm) of agar-salt solutions at two temperatures.

	EC	
% NaCl	53 C	20C
0.0	0.25 ± .01	0.15 ± .01
0.5	13.42 ± .25	
1.0	26.92 ± .23	13.25 ± .39
1.5	36.65 ± .10	
2.0	46.95 ± .18	22.85 ± .52
2.5	59.55 ± .17	
3.0	69.25 ± .14	
Water		0.03 ± .01

Table VII-5. IC50 values 1/ and associated standard errors for 15 alfalfa cultivars at seven levels of NaCl in agar.

Cultivar	IC50
Thor	1.43±.12
Ladak 65	1.41±.14
Vangard	1.31±.11
Vernal	1.46±.07
Olympic	1.24±.07
Apollo	1.36±.08
Drylander	1.43±.12
Ranger	1.31±.06
Grimm	1.42±.11
Anchor	1.51±.12
Iroquois	1.31±.09
Honeoye	1.36±.06
Riley	1.36±.13
Baker	1.51±.14
Salton	1.49±.11

1/ IC50 = the estimated inhibitory concentration of NaCl which prevents 50% of the seeds from germinating.

Agar (C-1), parental Ladak 65, and several germplasms were tested in agar containing six levels of NaCl for ability to germinate (Table VII-6). Seed from plants selected using the agar technique had a four-fold (3.75 times, adjusted increase) increase in germination over parental Ladak 65 at the 1.75% NaCl level. However, selection from blotters in germination boxes appeared to be ineffective in shifting the mean percent germination above that of its unselected parental population, Ladak 65. Agar (C-1) had twice the germination at 1.75% NaCl than other germplasms tested. This significant increase in germination at 1.75% NaCl indicates that ability to germinate in agar containing NaCl is heritable.

Response surface analyses (Table VII-7) indicate that the relationship between percent germination and NaCl concentration best fits a quadratic function. IC50's, although relatively smaller than those estimated for the cultivar survey, indicate that selection in Ladak 65 using agar has improved germination over the entire range of salt levels used (Fig. VII-2).

Selection for improved germination in saline antibiotic agar appears to be a heritable trait. Genetic variability among cultivars, marked response to selection at 1.75% NaCl, and a better resulting germination profile over a range of NaCl levels in the cultivar Ladak 65 indicate that the agar selection technique may be useful in breeding for salt tolerance of germinating seeds. The next step is to initiate additional

Table VII-6. Mean adjusted 1/ percent germination by level of NaCl in agar for seven alfalfa cultivars using four replicates for each treatment combination.

Cultivar	Percent NaCl (w/v)					
	0.00	0.50	1.00	1.50	1.75	2.00
Thor	100	97	90	20	5	1
Salton	100	96	96	34	5	1
Ladak 65	100	97	97	22	4	0
Blotter (C-1)	100	91	91	23	3	0
Agar (C-1)	100	100	97	39	15	2
994	100	88	88	24	6	1
Dryland composite (Dobrenz)	100	100	96	25	7	1

1/ Germination expressed as a percent of the 0.00 NaCl check within each cultivar.

Table VII-7. IC50's and associated standard errors for seven alfalfa cultivars at five levels of NaCl in agar.

Germplasm	IC50 1/
Thor	1.36±.14
Salton	1.43±.13
Ladak 65	1.39±.11
Blotter (C-1)	1.35±.15
Agar (C-1)	1.48±.10
994	1.33±.13
DO-1	1.41±.14

1/ Inhibitory concentration of NaCl which prevented germination of 50 percent of the seeds which would have germinated without NaCl present.

Figure VII-1. Alfalfa seed germination at 1.75% NaCl in antibiotic agar.

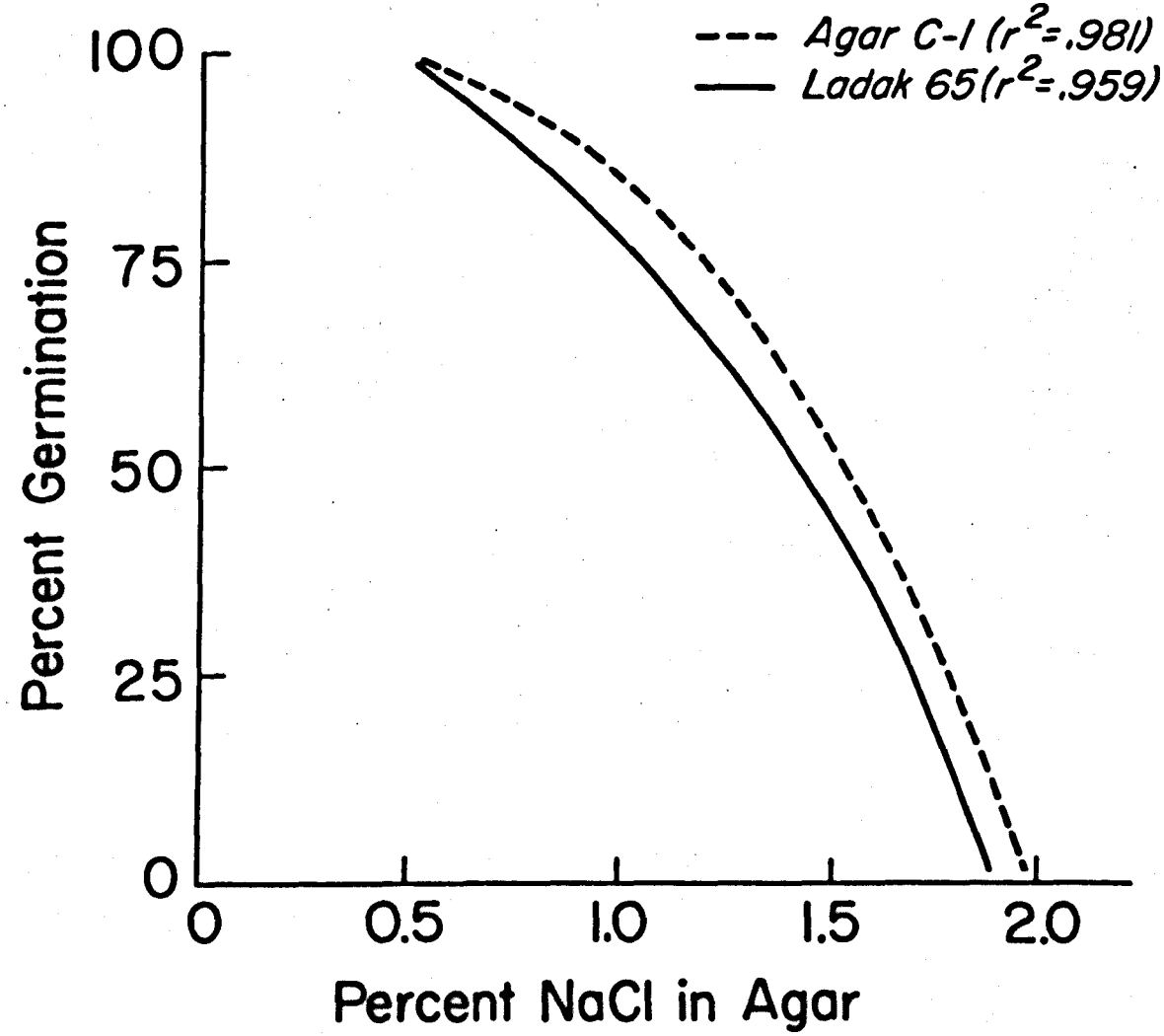

Figure VII-2. Quadratic response curves showing improvement in germination of Ladak 65 following one cycle of selection for ability to germinate in antibiotic agar containing 1.75% (w/v) NaCl.

selection cycles to determine the total improvement which can be realized. Improved cultivars will be tested over a range of saline environments. An improved cultivar with the ability to germinate to a greater degree in saline or dry soils could result in better stand establishment and thus improved yields. Marginal environments, previously of little agronomic value, could become more productive.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

Drought is one of the most important factors limiting alfalfa growth. It reduces yield and quality of alfalfa. The objectives of this study were to: 1) develop efficient screening procedures for physiological and morphological traits related to drought resistance of alfalfa; and 2) to determine if genetic progress through divergent selection can be made for these traits.

Traits evaluated were stomatal density, seedling root pattern, resistance to wilting induced by an artificial drought stress, leaf color, leaflet size, and germination salt tolerance.

Considerable within-plant variability for stomatal density existed. Variability among stems on the same plant necessitated the sampling of leaflets from an extremely large number of stems for detecting differences among clones. As a result, stomatal density was not used as a selection criterion for drought resistance in alfalfa.

The seedling root screening technique was repeatable but the traits (tap- or fibrous-rootedness) were lowly heritable. Following two cycles of divergent selection, mean root scores did not differ from the mean of parental Ladak 65, but did differ from each other. Breeding progress for seedling root pattern will probably be slow.

Divergent phenotypic selection during an artificially-imposed drought was used to study the variability in time of wilt occurring among Ladak 65 alfalfa seedlings. Both early and late wilt traits

were lowly heritable and plant size was negatively correlated to time of wilt. Larger seedlings wilted earlier and smaller seedlings were the last to wilt. Additional technique refinement is needed if this trait is to be successfully used to breed for drought resistance in alfalfa.

Large and small leaflet size and dark and pale leaf color were highly heritable after 2 years of field progeny testing. Rapid breeding progress for these traits should be realized.

Selection for alfalfa seed germination using NaCl in antibiotic agar was successful. The technique is repeatable and selection improved germination three to fourfold after only one cycle. Rapid breeding progress for this trait should be realized.

Future studies should involve: 1) further selection utilizing heritable, potentially-useful drought resistance traits to ascertain how much total improvement can be realized; 2) examination of other traits related to drought resistance which might prove useful; 3) studies relating heritable, drought resistance traits to field drought resistance; 4) study of the interrelationships of drought resistance traits; and 5) the combining of traits correlated with field drought resistance to produce alfalfa synthetics to eventually obtain superior dryland alfalfa cultivars.

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APPENDIX

Appendix table 1. Sources of germplasms and commercial cultivars used to assess variability in seedling root scores.

Germplasm or cultivar	Source
Erect (ER1)*	Cycle one selections for erect growth habit at Bozeman, MT in 1977
Prostrate (PR1)*	Cycle one selections for prostrate growth habit at Bozeman, MT in 1977
Dark (DK1)*	Cycle one selections for dark green leaf type at Bozeman, MT in 1977
Pale (PA1)*	Cycle one selections for pale green leaf type at Bozeman, MT in 1977
Large (LG1)*	Cycle one selections for large leaflet size at Bozeman, MT in 1977
Small (SM1)*	Cycle one selections for small leaflet size at Bozeman, MT in 1977
Regrowth (MR1)	Cycle one selections for rapid regrowth at Moccasin, MT in 1976
Nonregrowth (MN1)	Cycle one selections for slow regrowth at Moccasin, MT in 1976
Early maturity (EM1)*	Cycle one selections for early flowering at Bozeman, MT in 1977
Heat resistant (HR1)	Cycle one selections from Ladak 65 for desiccation tolerance in a 120 F oven
Wilt resistant (WR1)	Cycle one selections from Ladak 65 for resistance to wilt in conetainers at Bozeman, MT in 1977
Wilt susceptible (WS1)	Cycle one selections from Ladak 65 for susceptibility to wilt in conetainers at Bozeman, MT in 1977

(table continued)

Appendix table 1. continued

Germplasm or cultivar	Source
C-3	C-3 dryland alfalfa germplasm pool (Dr. C. E. Townsend, Colo. State Univ., Ft. Collins, CO)
C-6	C-6 dryland alfalfa germplasm pool (same as C-3)
Falcata	Composite of several Medicago falcata L. lines from the World Alfalfa Collection
"C-4"	Outcrossed progeny from a single, low photorespiring alfalfa plant acquired from Dr. A. K. Dobrenz, Univ. of Ariz., Tucson, AZ.
Anchor	North American Plant Breeders
Drylander	Canada
Grimm	Land variety
Kane	Canada
Ladak 65	Montana
Orenburg	Land variety
Ranger	Nebraska
Rhizoma	Canada
Thor	Northrup-King Co.
Vernal	Wisconsin

*Source populations for the above germplasms included the World Alfalfa Collection, C-3 dryland alfalfa germplasm pool, and a dryland variety trial.

Appendix table 2. Mean seedling root scores (SRS) of 55 TR2, 49 FR2, 8 TR1, 8 FR1, 5 TRG1, and 5 FRG1 progenies, and unselected Ladak 65.

Clone no.	No. of plants evaluated	SRS	Clone no.	No. of plants evaluated	SRS
TR2 progenies			FR2 progenies		
TR2- 9	28	3.90	FR2-57	25	4.80
TR2-66	21	3.85	FR2-44	14	4.50
TR2-20	9	3.79	FR2-48	27	4.47
TR2-42	37	3.75	FR2-39	32	4.44
TR2-29	21	3.74	FR2-55	24	4.40
TR2-24	27	3.73	FR2-11	16	4.37
TR2-61	23	3.70	FR2-42	34	4.29
TR2-34	35	3.67	FR2-28	34	4.27
TR2- 2	24	3.66	FR2-31	17	4.25
TR2-10	28	3.64	FR2-33	29	4.24
TR2-17	21	3.62	FR2-17	18	4.23
TR2-31	31	3.62	FR2-35	21	4.23
TR2-44	22	3.61	FR2- 6	18	4.17
TR2- 7	19	3.59	FR2-45	32	4.14
TR2-27	20	3.59	FR2-32	27	4.06
TR2-28	26	3.59	FR2- 2	22	4.05
TR2-59	21	3.59	FR2- 4	28	4.02
TR2-40	26	3.57	FR2-37	32	4.02
TR2-30	18	3.56	FR2- 5	21	4.01
TR2- 5	11	3.54	FR2-46	33	3.98
TR2-52	25	3.53	FR2-10	23	3.96
TR2- 3	12	3.50	FR2-50	42	3.94
TR2-25	8	3.50	FR2-54	30	3.93
TR2-55	24	3.49	FR2- 9	32	3.91
TR2-41	22	3.46	FR2-25	30	3.91
TR2-53	20	3.46	FR2-58	23	3.90
TR2-32	30	3.43	FR2- 3	21	3.89
TR2-18	15	3.42	FR2-19	25	3.89
TR2-48	21	3.41	FR2-14	36	3.88
TR2-26	12	3.37	FR2-30	11	3.83
TR2-43	17	3.37	FR2-34	17	3.81
TR2-45	26	3.36	FR2-27	24	3.78
TR2- 4	37	3.36	FR2-22	30	3.77

(table continued)

Appendix table 2. continued

Clone no.	No. of plants evaluated	SRS	Clone no.	No. of plants evaluated	SRS
TR2-39	21	3.33	FR2-40	20	3.77
TR2-54	26	3.33	FR2-41	25	3.76
TR2-16	31	3.32	FR2-16	15	3.75
TR2-22	26	3.30	FR2-36	24	3.75
TR2-11	30	3.29	FR2-24	26	3.72
TR2-47	26	3.29	FR2-47	31	3.72
TR2-21	24	3.26	FR2-51	18	3.72
TR2-15	26	3.22	FR2-43	31	3.70
TR2-50	17	3.20	FR2-18	20	3.62
TR2-58	19	3.17	FR2-12	23	3.60
TR2-14	26	3.07	FR2-38	24	3.58
TR2-38	23	3.03	FR2-29	19	3.55
TR2-65	23	2.99	FR2-26	38	3.52
TR2- 1	43	2.95	FR2-21	19	3.35
TR2-57	30	2.95	FR2-52	25	3.23
TR2-19	34	2.94	FR2-56	28	3.12
TR2-36	29	2.94			
TR2-33	21	2.85			
TR2-13	25	2.81			
TR2-56	37	2.81			
TR2- 6	13	2.77			
TR2-60	23	3.24			
Mean		3.36	Mean		3.95
TRG1 progenies			FRG1 progenies		
TRG1- 8	23	3.70	FRG1-28	30	4.05
TRG1-24	21	3.46	FRG1-29	15	4.03
TRG1-33	13	3.17	FRG1- 2	18	3.96
TRG1-29	17	3.16	FRG1-14	20	3.94
TRG1-12	23	2.88	FRG1-20	24	3.69
Mean		3.27	Mean		3.93

(table continued)

Appendix table 2. continued

Clone no.	No. of plants evaluated	SRS	Clone no.	No. of plants evaluated	SRS
TR1 progenies			FR1 progenies		
TR1- 8	23	3.68	FR1-45	32	4.35
TR1-24	25	3.58	FR1-42	27	4.22
TR1-30	35	3.47	FR1-29	22	4.02
TR1-CP	26	3.30	FR1-20	24	3.92
TR1-12	25	3.29	FR1- 2	24	3.75
TR1-29	38	3.29	FR1-CP	31	3.74
TR1- 2	23	3.07	FR1-28	19	3.73
TR1- 9	19	3.04	FR1-14	30	3.52
Mean		3.34	Mean		3.91
Ladak 65	96	3.67			

LSD (0.05) = 0.63
CV = 12.5%

Appendix table 3. Mean seedling root scores (SRS) using a 1-7 scale 1/ of several germplasms.

Entry	Number of plants evaluated	Mean SRS	Range
LG1-3	36	4.43	2-7
PA1-15	29	4.28	1-6
DK1-7	10	4.25	3-5
HR1	30	4.21	2-7
WR1-41	22	4.19	3-7
PA1-3	30	4.18	3-7
Salton	29	4.16	1-7
LG1-34	22	4.12	2-7
MN1-CP	32	4.09	3-7
DK1-42	19	4.00	3-6
PA1-23	23	3.99	3-5
Thor	28	3.96	2-7
"C-4"	26	3.92	3-6
LG1-12	12	3.90	2-5
SM1-14	24	3.83	3-4
MR1-31	20	3.82	1-6
MR1-1	32	3.71	2-4
MR1-16	22	3.68	2-5
Ladak 65	96	3.67	1-7
WR1-44	24	3.66	1-5
WR1-38	17	3.56	2-6
SA1-1	14	3.50	3-4
DK1-46	25	3.47	1-5
SM1-15	18	3.20	2-4
WS1-21	34	2.87	1-4

LSD (0.05) = 0.66
CV = 12.9%

Appendix table 4. Harvey's least squares analysis for day of wilt for 25 WS1 progenies, 4 WR1 progenies, Ladak 65, TR1 plants, and FR1 plants.

Source	df	SS	MS
Blocks	2	211.794	105.897
Progenies	31	5117.156	165.069*
B x P	62	4912.199	79.229
Residual	400	38157.453	95.394
Total	495	48363.813	

Appendix table 5. Harvey's least squares analysis of variance for day of wilt for 30 WR1 progenies, 2 WS1 progenies, and Ladak 65.

Source	df	SS	MS
Blocks	2	1792.367	896.184**
Progenies	32	3791.813	118.494**
B x P	64	2752.780	43.012**
Residual	529	12223.316	23.106
Total	627	20508.438	

Appendix table 6. Analysis of variance for metric ruler score combined over years in 1979-80.

Source	df	SS	MS
Blocks (B)	3	0.933	0.311*
Progenies (P)	53	139.556	2.633**
Years (Y)	1	9.275	9.275**
P x Y	53	11.488	0.217**
Error	321	34.666	0.108
Total	431	195.918	

**Significant at the .01 probability level.

Appendix table 7. Analysis of variance for visual color rating combined over years in 1979-80.

Source	df	SS	MS
Blocks (B)	3	0.427	0.142
Progenies (P)	55	96.476	1.754**
Years (Y)	1	0.397	0.397
P x Y	55	20.542	0.373**
Error	333	26.662	0.080
Total	447	144.505	

**Significant at the .01 probability level.