Ruby Valley pointvetch (Oxytropis riparia Litv.) karyotype, germplasm registration, and seed coat characterization

by Deborah Jean Solum

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
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Abstract:
Ruby Valley pointvetch (Oxtropis riparia Litv.) is a perennial forage legume with high salt and drought tolerance. However, pointvetch has a high hardseed content, which makes uniform stand establishment difficult. Studies were conducted to characterize the chromosome complement, register the germplasm, and identify the seed coat components. The chromosome complement of pointvetch is 2n=2x=16, and the chromosomes are generally smaller than other plants and animals.

The pointvetch germplasm was registered in 1990 and distributed upon request to researchers for further studies. The seed coat surface pattern and cell layer components were characterized as a basis for further studies involving hardseed mechanism(s). The seed components were similar to other related small seeded legumes, consisting of cuticle, palisade cells, hourglass cells, parenchyma, endosperm, and cotyledons progressing from exterior to interior. Four pointvetch seed lots were utilized, and all had similar exogenous and endogenous characteristics which indicated species stability in diverse environments. This is useful for taxonomic use in establishing evolutionary relationships.

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ABSTRACT

Ruby Valley pointvetch (Oxytropis riparia Litv.) is a perennial forage legume with high salt and drought tolerance. However, pointvetch has a high hardseed content, which makes uniform stand establishment difficult. Studies were conducted to characterize the chromosome complement, register the germplasm, and identify the seed coat components. The chromosome complement of pointvetch is 2n=2x=16, and the chromosomes are generally smaller than other plants and animals. The pointvetch germplasm was registered in 1990 and distributed upon request to researchers for further studies. The seed coat surface pattern and cell layer components were characterized as a basis for further studies involving hardseed mechanism(s). The seed components were similar to other related small seeded legumes, consisting of cuticle, palisade cells, hourglass cells, parenchyma, endosperm, and cotyledons progressing from exterior to interior. Four pointvetch seed lots were utilized, and all had similar exogenous and endogenous characteristics which indicated species stability in diverse environments. This is useful for taxonomic use in establishing evolutionary relationships.

CHAPTER 1

INTRODUCTION

Water stress and increasing soil and water salinity levels are adversely affecting food and feed production worldwide. Sedimentation and salinity are affecting water quality, agricultural production, and general land use in Montana (Water Quality Bureau, 1982). The Soil Conservation Service National Resource Inventory estimated in 1987 that 1.0 million ha of Montana land were eroded by water at a rate greater than normal (R. Nadwornick, personal communication). Additionally, the Water Quality Bureau (1975) reported that 2,944 km of streams in Montana were degraded by sediment, and 1,545 km were affected by salt. The development and utilization of plant species with high drought and salt tolerance as well as erosion control capabilities could provide a partial solution to these problems.

Ruby Valley pointvetch (Oxytropis riparia Litv.) is a perennial, small seeded legume which is a palatable livestock forage. Pointvetch also has potential for use in reclamation situations since it is highly drought and salt tolerant and has a prostrate growth habit for erosion control. However, pointvetch has a high hardseed content which makes establishment difficult in field conditions. The hard seed coat inhibits the uptake of water by the seed, which is necessary for germination, and is often the major factor causing hardseededness in legumes. The hard seed coat may be caused by genotypic and/or environmental factors.

Chromosome number and morphology are important in a genotypic study since they determine the ease of developing a line with uniform germination, increased salt or drought tolerance, and/or greater forage and seed production potential. Chromosomes are often characterized by an ideogram of a karyotype. A karyotype is the particular chromosome complement of an individual or a group of related individuals as defined by chromosome size, morphology, and number (Schulz-Schaeffer, 1980).

After the chromosome complement has been characterized and a seed source collected, the germplasm can be registered with the Crop Science Society of America and stored at a Plant Introduction Station and the National Seed Storage Laboratory. Seed is then distributed, upon request, to researchers for further study.

Seed coat characterization is necessary to properly identify components so further studies may be conducted to identify which components may be responsible for hardseededness by utilizing various environmental conditions and genotypes to obtain a wide range of hardseed percentages. Identified hard and non-hard seeds may also be compared for morphological differences to identify the hardseed mechanism(s). Some small seeded legumes exhibit visible seed coat component differences between hard and non-hard seeds while others do not.

Environmental conditions are an important factor in hardseed development. Hot and dry growing conditions during seed formation increase hardseededness in many crops, especially legumes. Generally, conditions which favor formation of small seeds also favor hardseed development.

The objectives of this study were to 1) verify the chromosome number and characterize the chromosome morphology and size, 2) register the pointvetch germplasm for breeding and experimental purposes, and 3) characterize the seed coat components for both hardseed studies and taxonomic comparisons.

CHAPTER 2

LITERATURE REVIEW

Ruby Valley Pointvetch Description

Ruby Valley pointvetch (*Oxytropis riparia* Litv.) is a member of the Leguminosae family in the Galegeae tribe with other genera such as *Wisteria*, *Indigofera*, and *Astragalus* (Smith, 1977). *O. riparia* is a deep taprooted, prostrate, perennial legume (Booth and Wright, 1966) with opposite leaves (Komarov, 1972) comprised of subsessile, oblanceolate to acutish leaflets (Barneby, 1952). Flowers are small, purplish, and papilionaceous, with a banner approximately 6 mm long (Barneby, 1952). Seed pods are nodding, stipitate, 10 to 20 mm long, and approximately 5 mm wide (Komarov, 1972).

The chromosome number of pointvetch is 2n=2x=16 (Astanova and Abdusaliamova, 1981; Schulz-Schaeffer et al., 1990). Astanova and Abdusaliamova (1981) also reported plants with 2n=4x=32. Ledingham and Rever (1963) reported that all *Oxytropis* species were either diploid (2n=16) or polyploid with multiples of 8 chromosomes.

Ruby Valley pointvetch is native to Russian Turkestan (Barneby, 1952) and was found growing in the Ruby Valley of southwestern Montana in 1930 (Green and Morris, 1935). It was originally named *Astragalus rubyi*

Green and Morris by Green and Morris (1935) and was reclassified by Barneby (1952) as Oxytropis riparia Litv. Pointvetch has been identified along the Green River northwest of Green River, Wyoming; along the Snake River on the Fort Hall Indian Reservation north of Pocatello, Idaho (Williams and Molyneux, 1988); and near Ice Slough, Wyoming (Lichvar and Dorn, 1982).

Benson (1954) reported that pointvetch grew well on alkaline soil. Eslick and Vogel (1959) determined that pointvetch emergence increased in soil moisture tensions greater than field capacity (-0.03 MPa) down to 7.5% soil moisture (-1.5 MPa). Delaney et al. (1986) reported that pointvetch had 90% germination in -2.0 MPa NaCl solutions while -1.0 MPa NaCl decreased alfalfa (Medicago sativa L.) germination 70%. Pointvetch has a higher NaCl toxicity tolerance and a greater tolerance to higher osmotic stress than alfalfa (Russo, 1985). Germination of O. riparia has been reported to be temperature insensitive between 5 and 35°C due to rapid germination potential (Townsend and McGinnies, 1972).

Toxicity is often a concern since two other species of Oxytropis contain swainsonine which is responsible for loco syndrome in livestock. O. riparia tested negative for swainsonine toxicity and contained no cyanide, soluble oxalates, or aliphatic nitro compounds (Williams and Molyneux, 1988).

The feeding value of pointvetch was reported as similar to the protein content, nitrogen-free extract, crude fiber, ether extract, and ash of alfalfa. However, pointvetch phosphorus content was higher than alfalfa grown in the same area (Green and Morris, 1935). Feeding studies involving lambs fed alfalfa hay compared to pointvetch hay showed similar results (Post, 1937).

Hardseededness

A high hardseed content is common in _O. riparia_ (Delaney et al., 1986; Hicks et al., 1987, 1990; Post, 1937). Delaney et al. (1986) reported up to 96% hardseed, and Post (1937) found 95% hardseed. Hicks et al. (1990) reported that mechanical scarification for 30 s in a Forsberg scarifier[1] (Forsberg's Inc., Thief River Falls, MN) reduced pointvetch hardseededness better than either sulfuric acid (H_2SO_4) or hot water bath treatments.

Hardseededness benefits the preservation of viability over extended periods of time. However, this may be a disadvantage in agricultural situations which require rapid, uniform stand establishment. Hardseededness is common in the Leguminosae family (Quinlivan, 1971) and depends on the genetic nature of the species as well as environmental conditions during seed maturation and storage (Hartmann and Kester, 1983). Grant Lipp and Ballard (1964) studied seed of subterranean clover (_Trifolium subterraneum_ L.) and concluded that large seed and soft seed had lower physiological dormancy than small seed and hard seed. Hardseededness in subterranean clover increased with growing season length and less variable temperatures (Quinlivan, 1965). Kowithayakorn and Hill (1982) reported that alfalfa hardseed increased to 87% when seed ripened during hot, dry weather, and

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[1]Mention of a specific brand, trade, or chemical name does not imply endorsement of that product over others of a similar nature or function.

decreased to 60% if seed ripened during cool, wet conditions. Additionally, crimson clover (Trifolium incarnatum L.) percent hardseed increased when conditions favored the production of small seeds (Crocker and Barton, 1953).

Seed Coat Morphology

Impermeable seeds or hardseeds are common in many families other than Leguminosae, such as Gramineae, Malvaceae, Chenopodiaceae, and Solanaceae (Barton, 1965; Rolston, 1978; Watson, 1948). Seed coat structure determines the degree of hardseededness in legumes. However, there is disagreement over which part(s) of the seed coat are responsible for impermeability (Watson, 1948). The general structure of a leguminous seed coat from the exterior progressing inward is cuticle, Malpighian cells (palisade cells), osteosclereid cells (hourglass cells), and parenchyma layer (nutrient layer) (Barton, 1965; Rolston, 1978; Watson, 1948). The cuticle and Malpighian cells are most commonly considered impermeable (Rolston, 1978). McKee et al. (1977) punctured crownvetch seeds to a depth of at least 98 μm to induce rapid germination. This depth included all of the seed coat components, aleurone layer, and part of the exterior endosperm. The crownvetch impermeable region included the cuticle and Malpighian cells, while the sub-Malpighian layers may have contained germination inhibitors which must also be disrupted to achieve maximum imbibition potential.

Other seed coat structures such as the hilum and strophiole may also be responsible for hardseededness (Hagon and Ballard, 1970; Hyde, 1954). The hilum is the funicular attachment point to the ovule. The hilar groove, located in the long axis of the hilum (Corner, 1951), affects imbibition by opening and closing in response to relative humidity (RH) (Hyde, 1954). The groove closes when RH increases due to swelling of the surrounding tissue and opens as RH decreases (Hyde, 1954). Rolston (1978) suggested the hilar groove acts as a hygroscopic valve, allowing moisture loss from the seed when ambient RH is less than the endogenous moisture content. Hyde (1954) reported that the groove began to function when seed moisture decreased below 25%, and desiccation below 14% moisture occurred only through the hilar groove. Up to this point all or part of the seed moisture was lost through the testa.

The strophiole is an external raphe enlargement related to seed dispersal (Rolston, 1978). Gopinathan and Babu (1985) described the strophiole as a seed coat area allowing water imbibition. They found that imbibition was not restricted when <u>Vigna umbellata</u> (Thunb.) Ohwi and Ohashi seeds were coated with araldite except for the strophiole region. However, seeds with only the strophiole zone covered with araldite did not imbibe. Hagon and Ballard (1970) reported similar results with subterranean clover. Miklas et al. (1987) determined that 98% of imbibition after mechanical scarification of cicer milkvetch occurred at the strophiole and seed tip, and 75% of imbibition after H_2SO_4 scarification was accounted for by the strophiole. Ballard (1976) reported that the strophiole became permeable after other parts of the testa were mechanically stressed, suggesting the strophiole and testa are an integrated water absorption system.

Electron Microscopy

Seed coat studies often utilized electron microscopy for seed component developmental and morphological research (Adams et al., 1985; Baker et al., 1987; Bragg, 1982, 1983; Bridges and Bragg, 1983; McDaniel, 1987; Trivedi and Gupta, 1987; Wolf and Baker, 1972; Yaklich et al., 1986, 1987). Lersten (1981) compared Leguminosae species in the Papilionoideae subfamily and found significant seed coat patterns between and within specific tribes, but could not conclude evolutionary trends. Trivedi and Bagchi (1982) reported similarities in testa structures within and between three tribes of the Papilionoideae subfamily. All of the Trifolieae tribe had one pattern while members of the Genisteae and Phaseoleae tribes had variable patterns. Even with these variations the authors concluded that the testa pattern was important for identifying species and establishing relationships.

Extensive soybean developmental studies have been conducted. Stages of soybean testa development were studied from early differentiation to seed maturity (Baker et al., 1987). Adams et al. (1985) reported on soybean seed protein body development, and Yaklich et al. (1987) examined pit and antipit structural changes in soybean seeds and seedling development.

CHAPTER 3

RUBY VALLEY POINTVETCH KARYOTYPE

Increasing levels of soil salinity and soil erosion have resulted in the need for the development of plant species to aid in control of these problems. Ruby Valley pointvetch is a highly salt tolerant plant species and has a prostrate growth habit which aids in erosion control. The plant carcasses from the previous growing season remain as a dense ground cover on the soil surface providing continual soil protection.

Information is limited on the development of pointvetch for forage and/or reclamation purposes. The genetic potential of pointvetch is not known and is necessary prior to initiation of a breeding program for an improved genotype.

The objective of this study was to identify the chromosome number and characterize the chromosome morphology of this species.

Materials and Methods

O. riparia seeds from accession PI 525497 (MT-101) (Solum et al., 1990) were collected from wild stands at Silver Star, Montana during October 1987 and were used for chromosome number and morphology identification. These seeds were scarified for 50 seconds in a Forsberg scarifier[1] (Forsberg's Inc., Thief River Falls, MN). Root tips were pretreated in ice water for 20 h, fixed in Carnoy's solution (3:1, 100% ethanol : glacial acetic acid) for 30 m, treated in 70 % ethanol for 10 m, and hydrolyzed in 5 N HCl at room temperature for 5 m. Root tips were Feulgen stained and macerated in 5 % aceto-carmine counter stain. Cells were observed with phase contrast microscopy. The relative chromosome length was expressed as the percentage of the total length of all chromosomes measured in the same cell to eliminate deviations due to differential contractions in individual metaphase cells. Chromosomes were numbered from the longest to the shortest. A total of thirteen cells was analyzed.

Results and Discussion

The chromosome number of O. riparia was confirmed to be 2n=2x=16 (Fig. 1). This is in agreement with Astanova and Abdusaliamova (1981), who also reported plants with 2n=4x=32. According to Ledingham and Rever (1963), Oxytropis species always have 2n=16 or are polyploids with some multiples of 8 chromosomes.

A karyotype of O. riparia is shown in Figure 2. Chromosome measurements indicate an average chromosome length of 3.2 μm. The average size of chromosomes in plants and animals in general is 6 μm with some exceeding 30 μm (Schulz-Schaeffer, 1980). Thus, the chromosomes of O. riparia are extremely small when compared to those of other species. Relative total chromosome lengths (RTCL) and short arm/long arm ratios (S/L) are presented in Table 1. One chromosome had near equal arm lengths (metacentric chromosome 7 : S/L