Winterhardiness of Pea

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INTRODUCTION

Winter survival of pea requires avoidance or tolerance to stresses which vary in nature and severity with production region and year. These stresses may include freezing temperatures, frost, frost heaving, waterlogging, and pests, especially diseases. Cultural practices impact the microenvironment in which the pea acclimates and overwinters. Thus, planting date, soil fertility, pest control, tillage, and residue management practices may affect both the ability of the pea to acclimate and the severity of stresses which must be tolerated. Laboratory tests can estimate overwintering ability of pea if representative of stresses in the field. However, winterhardiness of pea represents tolerance to a highly variable complex of winter rigours and thus may be poorly predicted from the reaction to a single stress.

METHODS OF ASSESSING WINTERHARDINESS

Field survival is the ultimate test of winterhardiness, but is often inconclusive due to annual variability in snow cover, fertility, soil moisture, and other factors which influence winterkill (13). Repeated field tests are needed to estimate cultiluvar winterhardiness within a production area (23). Furthermore, suitable winterhardiness is specific to the region. For example, the cultivar 'Fremus', which is considered winterhardy in France, had poor winter survival and was considered nonhardy in continental regions of the Pacific Northwest (5).

The "field survival index" developed by Fowler and Gusta (4) uses survival of standard wheat cultivars to index the severity of the winter stresses at each location. Survival of previously untested genotypes is adjusted according to the severity of the winter to give a standardized winter survival rating. Similarly, this method has worked well for untested pea genotypes (3).

Sites for evaluating winterhardiness should be selected or managed to provide sufficient stress to cause differential survival among the test genotypes. Standing stubble cut to different heights to capture different depths of snow, resulted in different severities of freezing stress (32). Raised and flat beds have been used to manipulate soil drainage and exposure to cool temperatures thus creating different levels of waterlogging and temperature stress (6). Using this technique in wet and dry regions provides additional separation of pea tolerances to winter stresses. Finally, winter pea with limited hardiness may be scored for injury symptoms following a specific low temperature event in the field. Distorted stem apices, leaves, and lesions at the stem base may be scored in the field, or from collected samples, to relate injury to specific freezing events (11).

METHODS OF ASSESSING COLD TOLERANCE

In areas where cold temperature most limits winter survival cold tolerance may be a good indicator of winterhardiness, and can be measured directly. Controlled freezing tests are simple, rapid, allow precise control over experimental conditions and facilitate replication of experiments over time (20).

To predict field survival, the controlled cold temperature stress must be similar to that in the field, and artificial stresses such as high intensity freezing or freeze desiccation must be avoided (30). In order to avoid supercooling, plants should be nucleated with ice crystals when tissue temperatures are slightly below their freezing point. Pea collected from the field generally have nucleating agents such as dust and bacteria on their surfaces, and are self-nucleating, while greenhouse and growth chamber grown pea may not have this ability. Following the initial freezing event, slow cooling ($<2°C/h^{-1}$) allows time for water redistribution within tissues and prevents intracellular ice formation during the later phases of the freezing test. Upon reaching the selected test temperature, plants are maintained at that value for 1 h to ensure temperature and moisture equilibration, and then removed from the freezer to thaw. Test temperatures of –8 to –12°C have allowed separation of winterhardy types from spring types (1, 3, 37). The longer the period for which the tissue is frozen, particularly if the temperature is close to the mean or median lethal temperature (LT_{50}), the greater the degree of injury (16). Swensen (36) found that evaluation of whole plant survival required at least 3 weeks of recovery in order to make accurate assessments in cold-hardened plants.

When both winterhardy and nonhardy cultivars were included, field survival was significantly correlated with percent survival following temperatures of –3, –6, or –9°C in controlled freezing tests ($r = 0.80$ to 0.94) (3). However, field survival of winterhardy cultivars was correlated only with the –9°C freezing test, and in only half of the field sites ($r = 0.57$ to 0.72). While cold temperature tolerance can be accurately measured under laboratory conditions, final selection for winterhardiness must be made under field conditions.

When cells are injured by freezing, selective permeability of the cell membrane is lost and metabolic functions are disrupted. Thus, freezing injury in excised plant tissues may be evaluated by testing the ability of cell membranes to plasmolyze in a hypertonic solution (30), or to retain solutes (8, 33). Impairment of metabolic functions may be assessed by measuring the ability of the tissue to reduce triphenyl tetrazolium chloride (TTC) (35). Of these three viability tests, the TTC and leachate tests are the most simple to perform. It should be noted that plants at different stages of cold hardening may respond differently to the viability assays (16), and that the relationship between viability assays and whole-plant survival must be established using a whole-plant freezing test.

Plant breeders required a simple, non-destructive method to assess the cold hardiness of plants in segregating populations. Ideally the method would involve a non-essential part of the plant, and would permit screening of hundreds of plants each day. Many changes occur during hardening (20) but it is difficult to determine which of these are causal. To date, controlled freeze tests and measurements of plant moisture content (see Plant Moisture section) offer the breeder the best means of predicting cold tolerance. Final evaluations for winterhardiness must still be done in the field.

GENETIC VARIATION AND SELECTION FOR COLD TOLERANCE AND WINTERHARDINESS

Winterhardiness in *Pisum* is a quantitatively inherited trait (4, 7, 22) and may be conditioned by as few as three genes or tight linkage groups. A small, but important, genetic range of cold tolerance exists in pea.

Cold tolerance of winterhardy pea cultivars 'Melrose' and 'Fenn', and accession ID2, is near –8.5°C, but spring cultivars are killed at temperatures near –4°C under the same acclimating field conditions (3, 36). This small difference allows commercial production of Austrian winter field peas in northern Idaho and adjacent areas (34).

Genes for winterhardiness were found to be closely linked to the dominant gene *Pl* on chromosome VI which conditions for pigmented hilum (21, 22), and to the recessive *er* gene on chromosome III which imparts resistance to powdery mildew (*Erysiphe polygoni*) (5). A few recombinant lines have now been obtained (D.L. Auld, pers. comm.) so it is possible to select lines winch possess different combinations of the traits. Genes for pigmented seed coat (*A*) and yellow cotyledons (*I*) on chromosome I are not linked to genes for winterhardiness, and the traits may be selected independently.

Choice of female parent was important for increasing winterhardiness (12, 21). Cultivar 'Tracer' was a better spring parent than 'Garfield' or 'Latah' in crosses with hardy parents for recovery of winterhardy progeny (21). Eteve (12) suggested that a cytoplasmic influence may exist for winterhardiness in peas. The ratio of general combining ability (GCA) to GCA plus specific combining ability ranged from 0.59 to 0.87, indicating that there was sufficient GCA in additive gene action of Austrian winter pea to allow successful selection for winterhardiness (4). Cultivar 'Melrose' and line ID2 were identified as good sources of winterhardiness.

PHYSIOLOGY OF COLD TOLERANCE

Little information has been published on the physiology of cold tolerance in pea. Inferences are often made from work with other winter annuals, such as winter cereals, in which cold tolerance is a transient feature and limited in its extent, similar to that found in pea.

Acclimation

The primary inductive stimulus for cold acclimation is cold temperature between 0 and 10°C. While some crop species are susceptible to chilling at these temperatures, pea seedlings are resistant to chilling in either dark or light (31). Acclimation has been reported in some nonhardy pea cultivars. The nonhardy pea cultivar, 'Alaska' as well as the winterhardy pea cultivar, 'Melrose', showed reduced post-thaw electrolyte leakage after 20 days in the dark at 2°C (39). Acclimation in the dark was inhibited by removing the cotyledons. However, the relationship of post-thaw electrolyte leakage to whole-plant survival was not determined. Post-thaw electrolyte leakage of 'Alaska' is similar to that of hardy pea cultivars, but 'Alaska' is neither as freezing tolerant (1), nor as winterhardy as 'Melrose' (3).

Fall acclimation of pea in the field is also dependent on temperature, although it may be influenced by other factors, such as residual soil nitrogen and waterlogging (19). High residual soil nitrogen reduced freezing tolerance 0.5°C and winter survival about 6%. Excessive precipitation and subsequent waterlogging reduced freezing tolerance from –7.5°C after 7 weeks of acclimation a value above -4°C when measured 2 weeks later. Similarly, survival of winter pea at a semi-maritime location near Corvallis, Oregon, USA with minimum temperatures of –4°C and excess soil moisture was significantly less than survival at a continental site near Weston, Oregon where minimum temperatures were between –8 and –14°C under relatively dry conditions (6). These studies suggest that waterlogged conditions may severely limit the ability of pea to acclimate or maintain cold hardiness in the field.

Plant Moisture

Reduced tissue moisture during acclimation is a prominent feature of winterhardy pea cultivars, but variable in nonhardy cultivars. Percent moisture declined in roots, stems, and leaves of winterhardy pea genotypes, 'Fenn' and 'ID-113', during acclimation under controlled conditions (37). When nonhardy genotypes were exposed to similar conditions, 'Alaska' epicotyls showed an increase in freezing tolerance and a decrease in percent moisture (39), while for 'Dark Skin Perfection' neither freezing tolerance nor tissue moisture were affected (37).

Variable field acclimating conditions result in variable freezing tolerance and tissue moisture. Freezing tolerance of winter pea acclimating in the field was significantly correlated with epicotyl percent moisture in the absence of waterlogging in each of two years ($r = 0.85^{**}$ and $r = 0.70^{*}$; *,** indicate $P < 0.05, 0.01$, respectively) (19) Under waterlogged conditions epicotyl moisture increased 2.2%, but freezing tolerance declined from –7.5°C to greater than –4°C.

Plant Dry Matter

Decreased moisture content during acclimation is a result of dry matter accumulating at a greater rate than moisture (19). Total dry matter accumulates in epicotyls of winterhardy pea seedlings at about the same rate as in nonhardy seedlings during acclimation in the field or under controlled conditions (37). Total sugars composed over 10% of the epicotyl dry weight in winterhardy pea cultivars, while significantly lower concentrations of 7.3% were detected in nonhardy cultivars after two weeks of acclimation under controlled conditions (1). Total sugars were correlated with survival when peas were exposed to –8, –12 and –16°C (r = 0.69**, r = 0.87** and r = 0.77**, respectively), but starch, reducing sugar and nitrogen concentrations were poorly correlated with freezing tolerance except at –16°C. Correlation coefficients for survival at –16°C with percent starch, percent reducing sugars, and percent nitrogen were 0.63**, 0.75**, and 0.60*, respectively. Similarly, Eteve *et al.* (10) found that the concentration of total sugars in hardy genotypes acclimated for 2 weeks under controlled conditions was maintained during further exposure to cool temperatures, whereas continued cooling of nonhardy genotypes resulted in a significant decline in total sugar concentration.

Solute accumulation increases the osmotic potential of pea tissues, lowers the freezing point, and reduces dehydration stress at any given freezing temperature. During 8 to 10 weeks of acclimation in the field, water potential of pea internode tissues decreased from -1.4 to -1.7 MPa in the winterhardy cultivar 'Melrose' (38). However, in the nonhardy cultivar 'Tracer' water potential decreased from -1.0 to -1.2 MPa, then increased to -1.0 MPa. At the end of acclimation the freezing point depression of 'Melrose' tissue was 2°C lower than that of 'Tracer' due to differences in solute concentration. However, freezing tolerance differed between the two cultivars by 5°C. This suggests that solute concentration accounted for nearly one-half of the difference in freezing tolerance between 'Tracer' and 'Melrose'.

Dry matter increased in cell walls of winterhardy 'Melrose' and nonhardy 'Alaska' seedlings during exposure to 2°C in the dark (39). The neutral sugar arabinose was largely responsible for this increase and occurred at twice the concentration in 'Melrose' as in 'Alaska' cell walls. Much of this sugar was incorporated into the glycoprotein 'extension', which contributes to the strength and rigidity of cell walls and is reputed in inhibit cell extension growth. Cell wall rigidity may be important in resisting tissue damage when ice masses are formed between cells during freezing and in providing support during thawing. Limiting cell extension growth may impact morphological development.

Plant Development and Morphology

Under acclimating conditions, cell extension growth appears to be more restricted in winterhardy pea cultivars than nonhardy cultivars, resulting in smaller leaflets (1) and reduced plant height (2). However, leaflet size within hardy types is a poor indicator of relative hardiness.

When grown in the field, winter pea has more branches and a prostrate rosette growth habit in contrast to spring pea with a single, upright stem. Branching and rosette growth have been suggested as good indicators of winterhardiness (2). However, hardy and non-hardy pea cultivars acclimated for up to seven weeks under controlled conditions of constant cool temperatures and short days did not develop branches (1, 37). Furthermore, freezing tolerance of upright, single-stemmed winter pea acclimated under controlled conditions was nearly equal to that of field-acclimated, rosetted, winter pea (–8.l and –8.6°C, respectively) (J.B. Swensen and G.A. Murray, unpub.). The prostrate rosette may aid winter survival of seedlings in the field by reducing plant exposure to cold temperatures, desiccation, and abrasive effects of wind, and by increasing contact with residual soil heat, especially with snow cover. While the rosette growth habit may aid overwintering field survival, the development of a rosette is not required for the development of freezing tolerance.

Development of branches may require either alternating cool and warm temperatures or a difference in root and shoot temperatures in addition to short days. In any event propensity to branch should be evaluated in field-acclimated seedlings.

In pea seedlings with normal leaf morphology, unexpanded leaflets and stipules protect the shoot apex from radiant cooling and resultant frost. The semi-leafless (*afaf stst*) pea line 'Baf' sustained more apical damage from frost than either the normal leafed winter type, 'Frisson', or the spring cultivar 'Rodogune' (12). This suggests that the semi-leafless trait in pea genotypes with intermediate winterhardiness may not be compatible with tolerance to cold temperature stress.

Photoperiod Responses

Two observations support the conclusion that delayed flowering in short daylengths and relative winterhardiness in pea may be related. Pea plants that flower under short days of autumn but before frosts are often killed or, if not, are severely damaged by frost after flowering (9, 11, 22). Plants selected from winterhardy x spring pea crosses that flowered under photoperiods of 12 h length or less, but had other characteristics similar to winter pea, were not hardy when field-tested (G.A. Murray, unpub.). It seems that pea genotypes that are unable to remain vegetative under shortening days in autumn are incapable of hardening.

A cotyledonary factor other than carbohydrates may also be related to acclimation. Cotyledon removal prevented acclimation of 'Melrose' in the dark (39). Cotyledon removal at an early age also influences flowering of pea (24). It would be interesting to correlate the flowering event with relative winterhardiness in pea with and without cotyledons and with different photoperiod sensitivities when grown at acclimating temperatures combined with different photoperiods.

CULTURAL FACTORS AFFECTING WINTERHARDINESS

Genotype, Seed Selection and Seedbed Preparation

Significant differences in winterhardiness and disease resistances exist in pea germplasm. Selection of genotypes with desirable overwintering traits needs to be combined with appropriate cultural practices for successful survival of winter stresses.

Vigorous, good-quality seed is required if peas are planted late to ensure exposure to optimum temperatures for hardening. With autumn-planted winter pea in the USA, the use of large and medium seed improved stand establishment and yield of late-sown pea, but not of those sown in early September (25).

Stubble remaining in no-till systems should provide overwintering protection and more assured moisture for peas in relatively dry climates. However, in wet areas of the northwestern USA. cool, wet soils have often reduced the productivity of overwintering pea in no-tillage fields (G.A. Murray- personal observation). Effective herbicides must be available if no-tillage practices are to be successful.

Planting on raised beds in moist, semi-maritime conditions near Corvallis, Oregon, significantly increased survival of hardy 'Common Austrian' winter pea and less hardy lines from Austrian winter x Early Perfection crosses, compared with survival of pea planted on flat ground (6). In more severe continental conditions near Weston, Oregon, survival was better with ground level plantings. These data again show the need to manage for avoidance of the most limiting winter stress; waterlogging in wet areas, cold temperatures in drier areas.

Sowing Date

Sowing date represents a paradox for agronomists. Early sowing of winter pea allows establishment of large, well-rooted vigorous plants which help to control erosion. These plants are less susceptible to damage from defoliating insects such as the pea leaf weevil (*Sitona lineatus*) (29), are more resistant to frost heaving, and produce larger seed yields than late-sown pea (28). However, early-sown pea are often poorly acclimated in the event of frosts. In fact, Dowker (9) planted pea earlier than the optimum date to increase differential winterkill between hardy and non-hardy types. Sowing time should he adjusted to allow peas to withstand the most important stress of the region. The period during which seasonal mean temperatures are 5 to 8°C in autumn is suggested for sowing winter hardy pea genotypes in France (11). In northern Idaho where exposure to cold temperature is often prevented by snow cover, peas should be sown in early September at temperatures warmer than those suggested by Eteve and Derieux (11) for optimum yield and winterhardiness (28).

Sowing Depth

Deep sowing is considered to be an important factor in increasing winterhardiness of most cool season grain legumes. But the rout crown must be formed at a depth which will not limit yield. Shallow-seeded plants frost-heave and so are more exposed to extreme cold but deeper seed plants often have the epicotyl broken during frost heaving leaving the cotyledons and roots underground. The later condition is almost always fatal while some frost heaved plants (entire plant above surface) can re-root under favourable spring conditions (G.A Murray, personal observation).

Sowing Density

Increased seedling density may reduce radiant cooling of individual stem bases and stabilize the boundary layer around the canopy, but supra-optimal plant density may decrease yield. However, high density sowing may be an appropriate practice in autumn;

to provide insurance against cold weather, when stands are normally reduced by freezing temperatures. The practicality and economics of such a practice should be evaluated in different circumstances. Optimum sowing densities and distances and distance between seedlings within- and between-rows vary significantly depending on semi dwarf vs tall growth habit and regional conditions.

Mixed Sowing Systems

Mixed sowing can improve the endurance of crop plants to cold temperatures. With winter pea - winter cereal mixtures, winter pea survival was increased from 66% in monocropped stands to 76% in mixed stands (27). In a related study, winter pea in mixtures with 25% winter cereals produced seed yields up to 27% greater than monocropped winter pea (26). Reduced incidence of *Sclerotinia* infection and improved light interception were thought to have contributed to improved performance of the winter pea in mixed stands.

FUTURE PROSPECTS

Cold tolerance values for spring and winter pea are remarkably similar; LT_{50} values vary between –4 and –10°C. Traditional breeding for significant improvements in cold tolerance by crossing hardy genotypes have not produced progeny with cold tolerance values greater than those of the parents Searches of germplasm for accessions with improved cold tolerance may prove equally unrewarding. Gene transfer from other plant species with more cold tolerance than pea may now be feasible and should be explored. Intergeneric crosses could improve the narrow genetic range of cold tolerance presently available.

Physiologists, plant breeders, and agronomists must cooperate in the difficult challenge of improving winterhardiness in future pea genotypes. Rapid, non-destructive, reliable methods for estimating cold tolerance of F_2 populations must be developed. Monitoring flowering responses of F_2 populations under short photoperiods while screening concurrently for other attributes (e.g. reactions to disease) may provide reliable estimates of acclimating potential in pea. Selecting for improved waterlogging tolerance may also improve overwintering ability in wet areas. Semi-leafless peas have been shown to have more waterlogging tolerance than normal leafed peas (18) but are more susceptible to frost (12).

Significant expansion of winterhardy pea production into traditionally spring-sown areas may depend on improved disease tolerance, and the adoption of cultural practices that allow better expression of existing cold tolerance and which afford greater protection against freezing. Mixed sowing with winter cereals warrants further investigation as a means of improving the overwintering ability of pea.

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