Evaluation of barley cultivars for gibberellic acid activity
by Eloise Lerch Barr

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
Montana State University

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ABSTRACT

Tests were run to determine the bioassay and extraction procedure most consistent and informative concerning GA activity. Acid phosphatase, α-amylase, and reducing sugar bioassays were tested. The α-amylase bioassay was chosen for the evaluation of the barley varieties. The "Tris" buffer and the organic solvent extractions were tested. The "Tris" buffer yielded more reliable and consistent information; therefore, it was chosen to be used for the barley cultivar evaluation studies. Twenty-five barley varieties were compared using the selected assay and extraction procedures. Nupana ranked first in GA activity, followed by 'Heines Flo', "Piroline', 'Klages', 'Betzes', and 'Horn' which are all classified as malting types. 'Freja' had the poorest GA activity, followed by 'Otis', Dekap', 'Compana', 'Hulless Compana', and 'Ingrid'. All of these cultivars are classified as feed types. The remaining 13 cultivars were mixed in their rankings between malting and feed types.

INTRODUCTION

Barley cultivars differ in their ability to germinate and utilize their endosperm food reserves. This variance results in certain barley varieties being designated malting types.

Rats fed malting-type barleys grew better than rats fed feed-type barleys during a feeding trial conducted at Montana State University. Malting-type barleys possess superior enzyme activities. However, it is doubtful that this should have produced the results observed since animal pancreatic enzymes probably are sufficient to digest the feed.

One promising theory is that the GA activity of malting-type barleys is greater. This higher GA activity would probably have the capability of causing the superior enzyme activity associated with malting-type barleys. The greater amount of hormone, however, would also mean a greater amount available for each of the various activities of gibberellins; therefore, the overall physiology of the seed would be improved.

Methods of determining GA activity rely on the hormone's capacity of producing results as measured by various assays. Bioassays are a quick and convenient method for determining the quantity or the presence of a particular plant hormone. The GA bioassay consists of applying the extracted material to a plant or plant parts and measuring the response. Results are most accurate

when one uses tissues which exhibit a narrow range of specificity for gibberellins.

There are numerous bioassays and gibberellin extraction procedures in the literature. The objectives of this study were to evaluate the published assay and extraction procedures for GA and to select one procedure to be used in the evaluation of 25 barley cultivars. Based on the results of these evaluations we would hope to explain why the rats fed malting-type barleys grew better than rats fed feed-type barleys.

LITERATURE REVIEW

Plant hormones known as gibberellins are intimately involved with organ development and growth at the cellular and organismal levels. Gibberellins function in the rearrangement of cellular organelles; in the stimulation of synthesis of various proteins; in stem elongation in brachytic dwarf plants; in the stimulation of parthenocarpic development of many fruits; and in the breaking of dormancy in plants such as grasses, carrots and lettuce (75).

Early gibberellin research centered on either the isolation of native gibberellins or on the gross effects that applied gibberellins had on the ultimate appearance of the treated seeds, flowers, or stems. Recently, the biochemical aspects of gibberellins, especially the molecular mechanisms of action have been studied.

Isolation of the various gibberellins required several decades of effort. Gibberella fujikuroi, the causative organism of Bakanae disease in rice produced a substance initially termed gibberellin B but later called gibberellin A. This substance induced the elongation of rice shoots, the identifying characteristic of Bakanae disease. Curtis and Cross (19), in attempting to isolate this substance, instead obtained a similar compound and termed it gibberellic acid (GA), which is also known as gibberellin A_3.

Approximately 38 naturally-occurring gibberellins have been isolated from fungal and higher plant substances (2, 35, 81). The

biosynthetic pathways leading to these gibberellins are still uncertain, but it is known that whenever radioactive acetate or mevalonate is applied to a test fungal system the label ultimately appears in the GA produced. Between the initial acetate and the final gibberellin, kaurine appears to be one of the intermediate compounds in the biosynthesis of gibberellins in both angiosperm and fungal systems (17, 18). Kaurinoic acid stimulates stem elongation in dwarf maize seedlings (30). Kaurine apparently undergoes an oxidative transformation first to gibberellins A_4 and A_7 and then to GA (79). The biological expression of a particular plant or seed may be based on its genetic ability or lack of ability to convert one gibberellin to another (24). A good example is Tan-ginbozu, a dwarf rice which has very little endogenous GA and yet shows various responses to a large number of applied gibberellins (24). Apparently the amount of activity may be due to the ability to fit a specific receptor site or to the efficiency of GA interconversion in the plant from inactive or less active GA's to active ones. In the natural situation when GA's are not applied, the small amount of native GA must be converted rapidly and frequently to supply the needed GA's. Not all requirements are fulfilled, hence the reason for the dwarfness of the Tan-ginbozu variety.

Brown (12) speculated that successful germination of barley depends on an interaction between the embryo and endosperm of the seed. He suggested that the embryo is the primary site of production of hydrolytic enzymes during germination since the stored carbohydrates present in a barley endosperm, separated from its embryo, failed to disintegrate. Another theory stated that although the embryo's presence was essential for the production of enzymes there was some unknown mechanism which caused the endosperm itself to be directly involved in enzyme production. This second theory of germination is now more widely accepted but not by everyone.

From 1897 to the early 1960's many researchers believed that the barley scutellum secreted vast amounts of hydrolytic enzymes into the endosperm (52, 53, 36, 44, 21, 22, 26, 6, 8). Since a larger scutellum would logically produce a greater amount of enzyme, a barley variety with a broad, circular scutellum was believed to be superior for malting production (52). The majority of those holding this view believed the epithelial cells of the scutellum were the primary site of enzyme synthesis. The debate ended when Paleg (57) showed that exogenously-supplied GA isolated from embryos was capable of causing endosperms, devoid of embryos, to disintegrate into recoverable fractions of reducing sugars. Some scientists still believe that the scutellum has major enzyme-producing power (11).

Once it was generally accepted that the scutellum was not the site of enzyme production the next significant finding was by MacLeod et al. (47, 48). They demonstrated that the aleurone layer of the endosperm was responsible for the formation of the starch-modifying enzymes. They found that enzymes such as α-amylase, endo-β-glucanase, and protease were synthesized when GA was supplied to barley endosperm pieces which still retained their outer coating of aleurone cells. They further asserted that the formation of endo-β-glucanase which is a cytase (attacking cell wall material) preceded the formation of α-amylase. This would definitely substantiate the theory that before any starch granules can be attached by amylolytic enzymes, the carbohydrates of the amyloplast wall must be partially degraded.

Natural GA present in the intact barley embryo (14, 49, 50, 70) migrates from the germinating embryo (62) to the aleurone layer (48). When this GA reaches the aleurone layer, it stimulates the production (71, 78) and later the release (37, 42, 63, 67) of hydrolytic enzymes. The enzymes degrate the endosperm, including the carbohydrates present in the cell walls, the protein matrix of the endosperm, and starch (61, 33). Theoretically, soon after obtaining a uniform distribution of hydrolytic enzymes in the endosperm (59), a complete modification of the primary structure of the endosperm occurs.

Several factors are needed for maximal seed germination of barley including water, oxygen and optimal temperature (15, 16). Varner et al. (77) showed that a continuous supply of GA to the aleurone cells is essential for germination. Therefore, the embryo of an intact seed must secrete a consistent amount of GA throughout germination. However, the literature (67, 37, 63) indicates that different hydrolytic enzymes require different amounts of GA to effect their maximal formation and release into the starchy endosperm. Slow germination of barley could result from inability of the embryo to secrete enough GA to the aleurone layer; a partial obstruction in the vascular system (62) which would reduce transport of the GA from the embryo to the aleurone; or an impediment of enzyme-formation response of the aleurone cells to the hormone (54, 38); any factor which decreases production of a particular enzyme (one which needed a high level of GA) could slow down germination.

Barley cultivars react differently to applied gibberellins. Excised 'Proctor' aleurone cells respond more slowly to exogenously-applied GA than do the aleurone cells of 'Kenia' or 'Zephyr'. In an intact system 'Proctor' responds similarly to 'Kenia' or 'Zephyr' (60) which would indicate that a greater amount of natural gibberellins in 'Proctor' compensates for the aleurone's lower level or response. These results suggest that certain cultivars contain a

greater amount of native gibberellins; the GA's of one cultivar have a greater potency than GA's of another cultivar; or the aleurone cells of some cultivars are just more responsive to exogenously-supplied GA than those of other cultivars.

Enzymes modify the endosperm first at the embryo end (60, 65). Even when GA is externally applied, this normal pattern of degradation is observed. Radioactive GA applied to intact seed enters the germinating seed via the embryo (59). This suggests that the pericarp of a barley seed is impermeable to ionic solutions. This property of the pericarp apparently prevents direct access of exogenously-applied GA to the underlying aleurone cells (60). With mechanical scarification of barley degradation occurs simultaneously "from both ends" (64). In test situation scarification allowed a greater surface area of the seed to be degraded at any one moment without a larger concentration of GA. Scarification also increased levels of α-amylase (59), endo-β-glucanase (72) and protease (60) without an increase in GA level.

Barley embryos secrete GA-like materials which induce aleurone cells to produce enzymes but the part of the embryo responsible for GA synthesis is not positively known. The scutellum may be a site of GA production as well as an organ for food storage and transport (58).

Radley (69) found GA_1 present in the seed after two days of germination. She suggests that this is an early form of GA and is located in the scutellum. After a few days of germination GA_3 formation begins in the embryonic axis. She found that excised scutella produced amounts of GA similar to that produced by the growing embryo. MacLeod and Palmer (49) in contrast to Radley's findings, isolated the scutellum from its growing embryo and attached it to an endosperm. No GA-like factors were either formed or transported to the attached endosperm. From their studies (49, 50) they designated the embryonic axis, consisting of the acrospire, the scutellar node, and the roots as the primary source of gibberellin-like factors. They indicate that the scutellar node and the roots were more important than the acrospire in causing the production of α-amylase in the aleurone. Radley (70) after further study substantiated this position and came to the conclusion that a scutellum devoid of its axis is capable of synthesizing gibberellins but is prevented from doing so by contact with an endosperm. She also stated that sucrose when applied externally to excised scutellum can inhibit gibberellin production. This observation is in contrast to the behavior of the intact germinating grain. In the latter system the scutellum synthesizes sucrose (25) even when the level of gibberellin is increasing (34). Radley, however, feels that the two phenomena

are mutually exclusive, and that the scutellum's sucrose-synthesizing ability does not interfere with its ability to synthesize GA_1, as long as the endosperm has been completely removed. This apparent paradox between gibberellins of isolated scutellum and gibberellins of whole grain has not been fully explained.

Cohen and Paleg (14) also attempted to locate the site of gibberellin produced in intact germinating barley embryos. They agreed with MacLeod and Palmer's conclusion (49) that the axis was of far greater importance than the scutellum in gibberellin production.

Another interesting opinion concerning the source of both gibberellins and initial enzymes is proposed by Briggs (10). He agrees with Radley (69, 70) that the scutellum is the site of production of GA and states that equal amounts of hormones are released to the ventral and dorsal surfaces of the seed. He also feels endosperm breakdown commences adjacent to the scutellum which indicates that the scutellum has produced both the GA's and its own enzymes. His view conflicts with that held by Brown and Morris (12) who based their opinions on a study of the time-course of endosperm modification. They concluded that the dorsal or non-furrowed surface of the seed became degraded much more rapidly than did the ventral surface. Years later, MacLeod and Palmer (49, 50) and Palmer (62) employed autoradiographic techniques and came to basically the same conclusion and

suggested that their "normally observed asymmetric pattern of endosperm gradation" was the direct result of the aleurone cells at the embryo end of the seed being stimulated by the arrival of GA before the aleurone cells farther away from the embryo.

A possible explanation for Brigg's observations (8, 10) that degradation begins adjacent to the scutellar epithelial cells rather than below the dorsally-located aleurone cells at the embryo end of the seed (49, 59) would be that the intermediate layer of crushed cell wall material that is located between the endosperm and the epithelial layer of the scutellum has a tendency to become dislodged during histological manipulation of germinated seed. Mistaking this open area where the crushed cell walls simply moved away from the epithelial layer for digested endosperm would lead to the assumption that the scutellum is emitting the enzymes responsible for the "degradation."

Another possible explanation for Brigg's observations (10) is that often after excision of the embryo or scutellum an almost imperceptible layer of aleurone cells remains in contact with the scutellum (49). This layer produces its normal complement of enzymes in response to the GA and the activity is mistakenly attributed to the scutellum. With careful excision and removal of the aleurone layer, the enzyme activity disappears (49).

The frequent contamination of the scutellum by aleurone was substantiated by Briggs and Clutterbuck (11) subsequent to Briggs' earlier observation (10). They further suggested that the epithelial cells of scutellum are indeed capable of producing α-amylase, but only in very minute quantities and only in the presence of externally-supplied amino acids. Where this speculation would fit into the picture of germination remains unclear. Work is definitely needed to fully elucidate the intricate interactions occurring between the growing embryo and the modifying endosperm.

There are many reports concerning GA's action on the aleurone layer of barley. The stimulatory capability of GA on aleurone cells is not its only function; its role in that system is sufficient to establish its physiological character as a dominant hormone in barley germination.

Although GA's are most responsible for the stimulation of enzyme production in the aleurone of barley, various other substances trigger α-amylase production. Some of these are suspected precursors of GA, but others apparently differ in biochemical origins. These substances include 3', 5'-cyclic AMP (23, 28) ADP (28) glutamate (29) asparate (29) ent-kaurine (39) helmenthosporic acid (9, 56) and several other compounds that are associated with the citric acid cycle (29). It is possible that GA stimulates the production of the various amino

acids (glutamate, asparate, etc.) which in turn stimulate the α-amylase synthesis. Based on this assumption the stimulation induced by the addition of these compounds is a later step in the pathway than the precursor stimulation. If this theory is correct, it could be proven by determining the length of time required for the various compounds to stimulate α-amylase; precursors of GA would take longer and amino acid stimulation would take less time than GA. Briggs and Clutterbuck (11) postulated that the ability of some of these substances to induce the production of α-amylase is based on endosperm starch being hydrolyzed by other enzymes typically found in barley aleurone.

Duffus and Duffus (23) demonstrated that a high concentration of cyclic AMP (10^{-3} μM) induced the production of a significant amount of α-amylase. A year later Pollard (66) found that GA enhanced the incorporation of radioactively-labelled adenine into adenosine 3', 5'-cyclic phosphate in barley aleurone cells. Therefore one mode of action of GA may be the induction or the enhancement of the synthesis of cyclic AMP-like compounds. Since stimulation of α-amylase by cyclic AMP is only 25% of the stimulation induced by GA, it appears that GA does considerably more than simply increase the cyclic AMP level.

Alpha-amylase production has long been considered a result of de novo synthesis (7) rather than an activation of preformed enzyme precursors. Using radioactive labelling and subsequent tracing and fingerprinting of derived proteins, Varner and Ramchandra (78) confirmed that both α-amylase and protease are synthesized de novo in the presence of GA.

GA may also trigger release of specific soluble RNA's (71). Since these same soluble RNA's are found in water-treated controls, it is possible that the constant requirement for GA in barley germination is for the "turnover" rather than the net synthesis of these soluble RNA fractions. No specific subcellular location has been linked to this GA-induced response.

GA's action on subcellular organelles (such as endoplasmic reticulum - ER - and polysomes of aleurone tissue) recently has been studied (27). Results indicate that GA stimulates the appearance both of ER and polysomes which are subsequently involved in enzyme synthesis. In the aleurone of wheat, a GA-like hormone is capable of inducing either the production of ER or the complexing of ribosomes with pre-existing endoplasmic membranes, prior to α-amylase synthesis (31).

Gibson and Paleg (31) suggested that α-amylase synthesis occurs in "lysosomes" of aleuronic endoplasmic reticulum (31) rather than

at free ribosomal sites as suggested by Varner and Ramchandra (78). This membrane-bound type of synthesis (lysosomal) of hydrolytic enzymes in barley aleurone layers would be logical since almost all the enzymes involved in the degradation of the starchy endosperm are produced and secreted from the aleurone layer. These aleurone cells retain their continuity and viability until all of the endosperm has been digested. Unless these hydrolases are structurally contained, by some type of membrane, there is nothing to prevent the aleurone cells from self-digesting. To further substantiate this theory Evins and Varner (27) reported a GA-dependent increase in membrane synthesis occurring at approximately the same time that the hydrolytic enzymes would be undergoing synthesis; therefore, the needed membranes would be present to encapsulate the new enzymes. Finally, electron micrographs by Vigil and Ruddat (80) have associated rough ER with the site of action and perhaps production of hydrolytic enzymes in GA-treated aleurone cells. Even though it has been reported that more than half the GA-induced α-amylase present in aleurone is "lysosomal" (31) it still cannot be unequivocably stated that either of these occurrences i.e., the synthesis of ER or the complexing of ribosomes with pre-existing ER, is the primary mode of action of GA.

The interactions occurring among the various plant hormones often makes interpretation of affects very difficult. For example, in some

deciduous woody plants, GA induces dormancy and yet conversely, in barley, high levels of GA appear to break dormancy (5). Abscisic acid (ABA) usually inhibits growth of stems and other plant parts, but it has a synergistic effect with GA that stimulates growth of rice mesocotyles (74). Since past work in the area of plant hormone activity shows a synergistic relationship between GA and auxin in various plant systems (5, 73) it is surprising that auxins (13, 51) play only a minor role in GA-induced responses in aleurone cells. In a few instances low levels of auxins may act to accelerate the GA-induced production of α-amylase in aleurone tissue (51, 80) but one investigator states that GA action on barley aleurone is independent of the presence or absence of IAA (13). Other authors claim a minor role for IAA in α-amylase production and suggest that IAA migrating from the coleoptile tip helps to lignify the cells in the scutellum (51) thereby hastening the construction of the vascular system which is theorized to be the transport pathway (62, 77) for GA's from the embryo to the aleurone. Supporting evidence for this theory shows that removal of the coleoptile decreases α-amylase synthesis. This decrease can be reversed by applying IAA (51).

GA is involved with the breaking of dormancy in several species of plants. Some investigations have suggested that the primary action of GA in dormancy breaking is due to the accelerated mobilization of

food reserves. The action of GA in barley contradicts this theory, since mobilization of food reserves of the endosperm succeeds rather than precedes germination (49, 58). More likely, the action of GA in dormant barley is a counter-balancing of an inhibitor such as ABA. ABA inhibits the natural response of aleurone cells to GA (37). This inhibition is a non-competitive interaction. Apparently, the ABA blocks formation of the subcellular organelles needed for enzyme synthesis (27). Ethylene, another hormone-like compound and GA together nearly eliminates the ABA inhibition. This might be explained by examining the system operating in peanuts. Ethylene is required for germination and ABA blocks ethylene synthesis - hence no germination. Ethylene then could be the unclassified "embryo factor" suggested in the literature (46, 51, 80) whose function would be to sensitize the aleurone to subsequent applications of GA. Although isolated barley aleurone responds to applied GA the secretion reportedly is far less than that achieved in the intact system (46). This would agree with the assumption that something is missing when a test is run in vitro.

If the system operating in barley is similar to cherry and hazel seeds than an increase in the number of adenine nucleotides is always associated with the breaking of dormancy (28). In hazel seeds (28) the GA_3-induced loss of dormancy also increases the incorporation of

adenine into AMP and ADP compounds. Many potentially rate-limiting steps occur in α-amylase synthesis that require adenine compounds. They can be actual precursors, activators, co-factors, or sources of energy. In any event, increasing adenine compounds would aid enzyme synthesis. High levels of GA (quantities on the order of 25 ppm) stimulate the rate of germination of barley (50). In other species GA induces elongation of dormant vegetative parts, and flowering of dormant buds by substituting for other growth-promoting conditions, such as light or cold temperature treatment (73). When excess moisture causes secondary dormancy of barley seeds, GA is ineffective in breaking dormancy (68).

One final interaction which may relate to the barley aleurone system is the effect of GA on the production of IAA oxidases. GA represses the production of these oxidases and thereby prevents the destruction of IAA. In this way both GA and IAA encourage cell division and elongation (75). These hormones also interact to create a greater osmotic potential in the meristematic regions. During competition between the vegetative and reproductive parts for water and nutrients the tissues with the higher GA content will act as sinks to attract both. Yields therefore depend on the GA-induced IAA available at the time of fruit set. The situation in barley may be analogous with head filling being a GA-IAA interaction. Therefore, a higher GA content would be advantageous during seed development.

MATERIALS AND METHODS

Determination of Bioassay Procedure

Seeds were prepared as follows for all bioassay evaluations: seed halves were incubated in a manner similar to that described by Jones and Varner (43). Nondormant Schuyler seeds were cut transversely and the embryo fractions were discarded. The endosperm halves were sterilized in a 1% sodium hypochlorite (commercial bleach) solution for 20 minutes and rinsed 10 times with distilled water. Groups of three seed halves were incubated in a 1.0 ml solution of streptomycin sulfate (100 µg/ml) and GA (10^{-7} M). Incubations were carried out in sterilized 6-dram screw top vials. At the end of the incubation period seed halves were removed from the incubation liquid and the liquid was tested for hormone activity.

Acid Phosphatase Assay The incubation solution in each vial was diluted to 5.0 ml with distilled water. Individual 1.0 ml portions were mixed with 1.0 ml portions of substrate solution. The substrate solution was prepared by mixing 30 ml of acetate buffer (pH 4.8, .1 M) 10 ml PNPP (50 mg. in 100 ml H_2O) and 1.0 ml of .1 M $MgCl_2$ (40). The mixtures were allowed to react for 30 minutes at room temperature, before adding 5.0 ml of 0.1 N NaOH. Optical density readings were made on a spectrophotometer Model PMQ 11, made by Carl Zeiss, Oberkochen/Wurtt., West Germany. The amount of phosphate produced was determined by absorbency readings at 410 nm.

Alpha-amylase Assay Following incubation an assay for α-amylase was conducted with 0.2 ml of the supernatant fraction plus 0.8 ml of H_2O as outlined by Jones and Varner (43). The reaction was started by the addition of 1.0 ml of starch substrate. The starch substrate was prepared by adding 0.15 g native potato starch, 0.6 g KH_2PO_4 and 200 μM $CaCl_2$ in a total volume of 100 ml distilled water. The mixture was boiled for one minute, cooled, and centrifuged for 10 minutes at 3000g. The reaction between the supernatant and the starch substrate was allowed to continue for 10 minutes. The reaction was stopped by the addition of 1.0 ml of an acid iodine reagent (prepared by combining 6 g KI and 0.6 g I_2 in 100 ml H_2O; 1.0 ml of this solution was added to 99 ml of 0.05 N HCl.). To this final reaction mixture 5.0 ml of distilled H_2O were added and the optical density was determined at 620 nm on the Zeiss spectrophotometer.

Reducing Sugar Measurement - Anthrone Reaction Two milliliter portions of the anthrone reagent (made by combining 0.2 g of anthrone with 100 ml concentrated H_2SO_4) (1) were placed in wide-mouthed test tubes and chilled in a water bath at 10 - 15C. One milliliter portions of the incubation medium were carefully added to the anthrone reagent and the mixture was allowed to cool. The test tubes were shaken vigorously while still immersed in the water bath. The samples

were removed from the water bath and brought to room temperature before being placed in a 90C water bath for 16 minutes. Samples were then cooled and read at 625 nm.

Determination of Extraction Procedure

Methanol Extraction The procedure of Mounla and Michael (55) was used. Three hundred seeds of barley were ground and extracted with 500 ml of aqueous 80% methanol for 24 hours at 4 - 8C and filtered. The filtrate was then evaporated to the aqueous phase on a rotary evaporator. The H_2O phase was adjusted to pH 8.5 partitioned two times against equal volumes of petroleum ether and then four times with equal volumes of ethyl acetate. The pH of the H_2O phase was reduced to 2.5 and the solution was partitioned four times with equal volumes of ethyl acetate. The acidic ethyl acetate was removed using a rotary evaporator. The medium was subsequently used as the incubation medium in the reducing sugar assay. The amount of reducing sugars produced were determined by measuring absorbence at 625 nm.

Buffer Extraction Three hundred seeds of barley were ground and suspended in 100 ml of 0.2 M "Tris" buffer (pH 7.2) following Jones procedure (41). The extraction continued for 3 hours. The solution was filtered and the filtrate was centrifuged for 25 minutes at 15,000g. The supernatant was treated with 0.4 N HCl to hydrolyze any "bound" GA which might be present and to precipitate the protein.

The hydrolysis continued for 30 minutes after addition of the acid at 35C using a mechanical stirrer. Following precipitation of the protein, the extract was again centrifuged for 30 minutes. The pH of the supernatant was adjusted to 2.5 and partitioned three times against ethyl acetate. The acidic ethyl acetate fraction was reduced to dryness on a rotary evaporator. The extracted medium was tested for GA activity with the α-amylase bioassay.

Evaluation of Barley Cultivars for GA Activity The buffer extraction and the α-amylase bioassay were used for the evaluation experiment with the following changes: for each of the 25 varieties, a total of 300 seeds were used, giving 75 seeds for each of the four replications. The ground seeds were extracted with 25 ml of "Tris" buffer (pH 7.2) as stated. The embryoless seeds were imbibed on sterile sand in 10 cm Petri dishes each containing 100 g sterilized sand and 20 ml sterile H_2O. Following a 72 hour imbibition period, 10 embryoless seeds were transferred to 25 ml Erlenmeyer flasks containing 20 μM CaCl2 and the extracted solution. The flasks containing the incubation material were autoclaved before incubating for 24 hours at room temperature.

An analysis of variance and a Duncan's Multiple Range test were used to determine statistical differences and for mean separation.

RESULTS AND DISCUSSION

Determination of Assay Procedure

Acid Phosphatase Assay: Due to unknown reasons insufficient inorganic phosphate was obtained in any of the trials to give adequate readings on the spectrophotometer so this test was not used for the barley evaluations. Since phosphatase is normally present in aleurone walls of dry seed it should be an early-release enzyme when GA is applied. No definite incubation time was stated by Jones (40) so possibly the time chosen in this test was incorrect.

Alpha-amylase Assay: This assay showed good repeatability (Table 1) and has some advantages over the other assays: 1) it is highly specific in its response to GA as neither auxins or cytokinins have any apparent action on the system, 2) production of α-amylase is closer to GA action than is the production of reducing sugars and, 3) it is not susceptible to contamination by micro-organisms which could cause false positive readings as in the reducing sugars assay.

Reducing Sugar Assay: This assay showed good repeatability (Table 1) but has several disadvantages: 1) it is farther away from the direct action of GA so any procedural error will be magnified, 2) it can be contaminated by the presence of micro-organisms causing false positive readings and, 3) any organic solvents present, including ethyl acetate can give positive readings. Even though the repeatability of the

TABLE 1 Mean absorbancies and confidence limits (.05) for two different gibberellin bioassays with embryoless barley seeds.

Alpha-amylase Bioassay[1]

Commercial GA	Buffer Extraction of GA from seed
.552±.034	.259±.029

Reducing Sugar Bioassay[2]

Commercial GA	Organic Solvent Extraction of GA from seed	Acidified water and Organic Solvent
.607±.033	1.295±.039	.243±.099

[1]Bioassay based on disappearance of starch-iodine complex.

[2]Bioassay based on production of reducing sugars.

reducing sugar assay was similar to the α-amylase assay, it was not used due to the many disadvantages stated.

Determination of Extraction Procedure

Organic Solvent Extraction Results: Residues left in the system from the organic solvents made this extraction procedure undesirable (Table 1). When an organic solvent was added to acidified water an absorbence reading was obtained (Table 1). Although the absorbency readings were consistent it was not reliable to simply subtract a certain amount and consider it attributable to the residues, since there is no reason to believe the amount of residue left is constant.

This extraction takes considerably longer than the buffer extraction and the use of ficin to hydrolyze protein-bound GA's is very unsatisfactory, since it's capable of carrying out other hydrolytic reactions. Also the ficin is used late in the extraction procedure at a time when the protein-bound GA's would have already been precipitated out by the organic solvents. As a consequence of these shortcomings, this extraction was abandoned.

Buffer Extraction: This extraction procedure is repeatable (Table 1) and highly consistent and has several advantages: 1) it eliminates the use of residue-leaving organic solvents, 2) it requires considerably less time than the organic extraction and, 3) it is capable of yielding a superior amount of GA due to a greater ability of the

system to hold protein-bound GA's. This allows a more complete recovery of all endogenous GA's. This extraction was chosen to be used for the barley evaluations.

Barley Cultivar Evaluations: Twenty-five barley cultivars were evaluated for GA activity using the buffer extraction procedure and the α-amylase bioassay. These evaluations show that the 25 barley cultivars vary greatly in their GA activity (Table 2). 'Nupana' was found to have the greatest GA activity followed by four barley cultivars which are considered malting types. They are 'Heines Flo', 'Piroline', 'Klages', and 'Betzes'. 'Freja' had the lowest GA activity. The feed types - 'Otis', 'Dekap', 'Compana', and 'Hulless Compana' were the next four lowest. The middle group is a mixture of malting and feed types; however, many of these cultivars are not statistically different.

The lack of a hull on Nupana was not the reason for its superior performance as Hulless Compana, an isogenic line of Compana, was almost identical to Compana being one position higher in the ranking. This one position could perhaps be ascribed to the hulless characteristic but Nupana's vastly superior performance could not.

Nupana was shown by J. Brown (personal communication) to have a superior respiratory rate than many of the varieties evaluated in this study.

TABLE 2 Mean values of absorbance readings for 25 varieties of barley[1]

Nupana	.06	a
Heines Flo	.12	b
Piroline	.18	c
Klages	.19	cd
Betzes	.22	de
Horn	.23	ef
Unitan	.26	fg
Munsing	.27	gh
Vantage	.28	gh
Hannchen	.29	gh
Heins Hanna	.29	gh
Traill	.29	ghi
Spartan	.30	hij
Svalof	.32	ij
Steptoe	.33	jk
New Moravian	.36	k
Brunes Wisa	.42	l
Haisa	.45	l
Firlbecks III	.45	l
Ingrid	.45	l
Hulless Compana	.51	m
Compana	.57	n
Dekap	.59	no
Otis	.62	o
Freja	.71	p

[1]Values expressed are an average of 12 observations. Means bearing different letters are significantly different (p .01) as determined by Duncan's Multiple Range test.

Various starches and various barleys have different rates of digestion (20). The amylopectin component of starch is digested significantly faster (p .01) than the amylose component; followed by endosperm material from two varieties of barley; and the slowest was corn starch. Therefore it is possible that the starches of malting barley types are more easily digested, allowing for increased utilization of the available food.

The proportions of amylose and amylopectin in various barleys differ (32). Malting barley cultivars may have a greater amylopectin component which is more readily available to the animal. Hence the malting types would actually be the better feed types.

An interesting comparison between Goering's rankings (32) of amylose values and these results comes from observing Dekap. It is a feed-type barley and held the 22nd position in this study. Compana held the position immediately above it with 'Spartan' somewhat higher. In Goering's ranking, Dekap had a lower proportion of amylopectin (the readily available starch component) than Compana, Spartan, and several other varieties. Although the differences were not significant, these findings do lend support to the theory that a higher GA activity leads to a greater amylopectin component and hence, easier digestibility. The increased activity of GA in malting type barleys may be conducive to the formation of more

easily digested starch and certain important proteins, namely enzymes specifically aimed at more complete starch degradation. Since hormonal mechanisms and interactions are not well understood this is entirely possible.

SUMMARY AND CONCLUSIONS

The α-amylase assay was selected as the bioassay to be used in the evaluation of barley cultivars for GA activity. It showed good repeatability and had numerous advantages: 1) it is highly specific in its response to GA as neither auxins or cytokinins have any apparent action on the system, 2) production of α-amylase is close to GA action than is the production of reducing sugars and, 3) it is not susceptible to contamination by micro-organisms which could cause false positive readings as in the reducing sugars assay.

The buffer extraction was chosen to be used in the evaluation of the barley cultivars since it was consistent and lacked the detrimental factors of the organic solvent extraction.

My data show that malting barleys have a superior GA activity. Of the 25 varieties tested Nupana ranked first in enzyme and hormonal abilities followed by Heines Flo, Piroline, Klages, Betzes, and Horn. These top six were malting type barleys with the exception of Nupana which has not been evaluated for malting quality. The bottom six cultivars having the lowest GA activity are classified as feed barleys. The chosen laboratory techniques proved consistent and informative in this evaluation study.

The varieties tested are currently being evaluated for speed of germination, seedling elongation, and effect of prechill. Results from this study will be evaluated in relation to GA activity.

Varieties whose embryonic organs differentiate early develop best in the field. Perhaps speed of germination and elongation will be related to GA activity and will be a simple technique for selecting superior types.

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