GERMINATION - A REVIEW

Germination continues to be one of the major problems in the development of irises of the aril and arilbred groups. Altho much experimentation has been conducted on germination by various aril workers relatively few have produced results which have been published. In a recent article in the American Iris Society Bulletin (April 1966) Mr. Kenneth Kidd has comprehensively reviewed the iris literature on this subject and appended a substantial bibliography. In the discussion he has mentioned the early work by Randolph and Cox in this country and Dr. Peter Werckmeister in Germany. He also discusses Dr. Lee Lenz' work on aril embryo culture and cites two recent articles by Werckmeister. His review is recommended reading for all.

In aril work we encounter many problems which are perhaps more troublesome than those found in other groups of bearded irises. Genetic factors play a part in germination, and in aril and arilbred work these are of importance. The germination capabilities of some bearded irises are quite high approaching the 90% range for T.B.'s on embryo culture. This contrasts with germinabilities of from none to almost 100% in the aril and arilbred fields. There are many references to germinability in Aril Society literature most of it coming in reports from embryo culturists. While we rarely have comparable information about natural germination we usually assume that types of seeds whose embryos germinate on agar and which have apparently adequate and normal endosperm will germinate naturally with proper handling. That natural germination may be exceedingly protracted is too well known for much comment. The assumption that nice, normal appearing seed will germinate naturally is not necessarily true — such seed may have no viable embryos due to genetic factors. It also does not follow necessarily that seed which have apparently normal endosperm and apparently normal embryos will germinate either on agar or naturally. Fortunately however, this last is not the rule. Failure of embryos to germinate on agar even tho of normal appearance has been frequently reported, especially from seed of crosses involving some regelia blood. Since embryo culturists rarely try natural germination of some seed from the lots they culture it is impossible to know if these embryos would have germinated on a different media or different handling or whether the seed would have germinated naturally on planting.

Criticism has been extended towards germination work done without proper "controls". In aril and arilbred work the problem is complicated by a number of factors. Embryo culture is perhaps the best control we have for assessing germinability. Here, however, judgment and even philosophy enters the problem. There is reason to believe that not all germinable embryos germinate on agar since the composition of the agar, its method of preparation, genetic factors and others have an influence. Also, we have the problem of germination anomalies. These include the often reported "all roots and no tops", "all tops and no roots", and embryos which grow and appear to germinate but which exhibit arrested growth rather early in comparison with some of their pod siblings, and finally die. We might perhaps evaluate the group on an "apparently normal" basis but this is a subjective judgement and would vary with the experience and insight of the observer. It is felt that this may be an adequate evaluation where such work is done to estimate the probability of getting reasonably good germination by "enlightened" handling.

Another problem in our field is that seldom do we have enough seed for controls to mean too much. From 20 seed of a cross how many could we af-

ford to use as a control? Even if we sacrificed half the seed, would a "controlled" germination test give statistially significant results? For embryo culturists, who follow the practice of embryo culturing a portion of a cross or lot of seed to check the condition of embryos and amount and appearance of the endosperm as a prelude to the decision whether to plant or embryo culture the balance, controls are no problems. At this writing despite some of the problems mentioned above, either examination of the embryos or better examination with culturing of the excised embryos are the best controls we have at this time.

We have some evidence that certain embryos which normally do not germinate on agar may germinate on natural planting. One of these groups is that of seed from crosses of C. G. White hybrids x onco or regeliocycli. Many of such seed are of regular appearance, of normal size but have a tiny spherical shaped embryos (Loveridge, Foster, Doran and others, Aril Society News-Letters, various). These "Pro-embryos", "immature embryos" or whatever they may be correctly designated have been thought to be ungerminable on agar. However, it has been reported that such embryos germinate on media containing "Super-thrive" a proprietary plant hormone preparation of unstated composition (Rich, Private communication 1965). Since such miniature embryos are turgid, of normal appearing tissue the definite possibility exists that these might germinate naturally under favorable conditions.

A few years ago the Species Committee received a large lot of I. **sofarana** seeds. After a number of reliable embryo culturists reported low or no germination some 50 seeds were sent to the Boyce-Thompson Institute, thru the kind auspices of Dr. Lela Barton. This institute is world reknowned in the field of seed germination, one of its principal fields of endeavor. The seed were tested for germinability by Dr. Florence Flemion. Dr. Flemion cautiously suggested (due to the limited number of seed available) that the difficulty appeared to be caused by micropylar contamination of the embryos by fungi or bacteria. She stated that the institute had found many instances of this in quite a number of species and genera. Such contamination has not been reported from garden pollinated aril or arilbred seed. The structure of the micropylar region plus the fact that a layer of tissue only a few thousands of an inch thick protects the embryo from the contaminating influences of its environment after dehiscence makes such a thesis extremely probable. While such contamination would almost certainly be fatal to embryos germinating on agar it might not be to a seed germinating in the soil. One always suspects bacteria or fungi of being inevitably enemies — however, some play a beneficial role to our plants. With garden pollinated seed such micropylar contamination should be rare. However, the traditional method of rolling freshly harvested seed in semesan or other mercurials (which originated in cool areas of high humidity) may have developed to prevent such contamination during storage. Garden pollinated onco species seed usually germinate in excess of 90% on agar, whereas collected seed vary very erratically. Shockey (1963) has suggested that seed collected from the ground may have been killed by over-exposure to the high surface temperatures of the habitats.

Normal planting as usually conducted would seem to be a very poor control for evaluating germination techniques. Birds, soil bacteria, and fungi, and the insect life of the soil may take a fearful toll of seed unless these factors are taken into account. The "Robins" have reported numerous instances in which the media in which seed were planted was screened and many seeds were either found missing or only the seed coats remained. The

late Herbert Kerr wrote in an early news-letter of finding almost microscopic white worms in some of his planted seed and recommended the use of dieldrin at appropriate intervals to eliminate such soil insects. Other insects attack the growing germinating seedlings prior to emergence, and earwigs are notorious for amputating tiny seedlings. Aside from such items there are too many variables involved in natural germination for it to be too good a "control", to evaluate new methods too realistically. Despite other factors excision of the embryo and examination, either with or without subsequent culturing on agar, is considered greatly superior to normal planting for the estimation of germinability.

Most of us are aware that there are seeds from certain types of crosses which can hardly be handled successfully except by embryo culutre. These include seeds with little or no endosperm and seeds with soft endosperm. These are often found from 2n x 4n and vice versa crossings, and in other wide crosses. The late Jack Linse advocated the "SQUEEZE TEST" for detecting seeds with soft endosperm. The embryos of such defective seed are expelled thru the micropyle by squeezing the seed with moderate pressure of the fingers at harvesting. Grossly excessive pressure of course will expel the embryos from some normal seeds. Our best present evidence is that seed with soft endosperm can only be handled successfully by embryo culture, and that such endosperm is usually lethal to the embryo unless it is relatively promptly excised. In making notes on seed production some report seed as apparently normal or good, fair, and poor or doubtful. Altho arbitrary it is felt that such practices are of merit.

It is fairly definite that all aril species have inhibitors in the endosperm. It is quite definite that the hexapogons additionally have inhibitors in the embryos. (Werckmeister 1952; Lenz 1955). Reports from embryo culturists indicate that embryos from seed with regelias in their parentages often fail to germinate on agar. Lenz (1955) recommended refrigeration to break such dormancy. Shockey (1959) recommended a formula containing glucose, maltose and dextrins as well as the customary sucrose as effective in breaking such dormancy without refrigeration. He attributed the effectiveness of this media to the simple sugars. In a later publication (1963) he discussed the metabolic requirements of the germinating embryo on agar. He recommends a newer media which contains fructose as well as glucose. His discussion mentions the role of fructose in the formation of the very important hexose phosphates which figure prominently in some of the bio-chemical transformations of plant cells. The implication that the carbohydrate metabolism of the germinating embryo is involved in the dormancy of the hexapogon embryos is far reaching and not fully understood. Oddly enough much of the phenomena encountered in dormancy and its breaking is very little understood i.e. cold treatment. Altho the facts are well known that cold treatments are almost universally successful in breaking dormancy in seed work, there are as yet no very satisfying reasons as to why they work.

Some seeds have coats which are impervious to the imbibition of water and to the diffusion of oxygen thru the endosperm into the embryo. Altho not published as such there is much information to indicate that moisture moves readily thru the endosperm to the embryo, and that this is not a problem in the germination of aril iris seeds. It is, however, not certain whether oxygen diffuses readily thru the endosperm or that carbon dioxide diffuses readily outward. In some seed it has been found that ready permeability of the endosperm to moisture is not necessarliy indicative that there is also ready diffusion of gases thru the endosperm. Respiratory studies of the

germinating embryo during after-ripening, both on agar and in the seed will be needed before aril germination can be understood.

In the 1963 First International Iris Symposium Max Steiger reported a new method of seed culture which he utilized in his work on I. **Kaempfiri.** Attempts to use this method with aril and aril hybrid seed have been unsuccessful. Several of the variations described by Steiger, such as pre-preparation of the seed by the Cluff method, were also unsuccessful. News-Letter reports on attempts to utilize the Cluff method on aril and aril hybrid seed indicate that one of the drawbacks of this plate culure method was the too common tendency of the embryos to abort thru the micropyle. This difficulty has also been found with one variation of the Steiger method. Analysis of embryo culture indicates two important factors which operate to make it effective: First the embryo is removed from the endosperm and contact with its inhibitors into contact with a reasonably adequate source of nutrients for its growth; and secondly the embryo is moved into an environment with relatively free contact with the oxygen of the air. Attempts are being made to develop a modification of such methods by cutting small openings into the seeds to expose the embryos to some oxygen, yet under such conditions that the embryos cannot abort. A few plants have been germinated by all the above methods. However, we cannot overlook the fact that the inhibitors and other "germination" blocks are present in varying degrees in all lots of normal seeds. As a result a few seed from almost any lot of onco or onco hybrid seed will usually germinate in a reasonable period of time with anything approaching intelligent handling. If this new surgical approach creates reasonably prompt germination generally with our aril seeds it will indicate almost conclusively that aril dormancy is indirectly due to lack of oxygen permeability of the endosperm. Since the embryo is still in contact with the inhibitors of the endosperm this would be highly indicative that the inhibiting effects of these are eliminated by oxidation and possibly other transformations.

Randolph and Cox (1943) demonstrated that the endosperm of iris seeds contained substances which inhibited the germination of iris embryos on agar. Other workers have shown that this is rather general in irises. One of Randolph and Cox's experiments used dripping tap water to leach chipped seeds and secured substantial germination in 5 weeks. This experiment indicated that some of the inhibiting materials were water soluble, but also that there was a fairly persistent substance or group of inhibiting substances which were difficult to remove. Jorgenson (1965) reported that the inhibitors in iris endosperm (T.B.'s) were of two types. One a substance or group of substances which were water soluble. The other a substance or group of substances which were soluble in ether. Isolated experiments have been reported using leaching to remove inhibitors in onco seeds, and leaching followed by after-ripening. Some germination was found but the experiments involved too few seed to be other than indicative that such methods might be worth following up. The existence of both water soluble and ether soluble inhibitors is of interest. Crocker and Associates (1953) discuss changes in the endosperm on after ripening. As a generalization on after-ripening the storage nutrients of the endosperm are degraded usually by enzymatic action to simpler more soluble materials. It is quite possible that the ether soluble germination inhibitors may be degraded on after-ripening to water soluble materials which are eliminated by leaching or metabolized. In nature something certainly removes or alters such substances.

Iris enthusiasts have always had an interest in germination stimulants.

That none have so far proven too effective is not surprising. Until there is knowledge about the chemical nature of iris inhibitors, how they inhibit, and how they are disposed of to permit germination there is no rational basis for selecting materials for germination stimulants. Kidds review covers the ordinary ones which generally have been of little use for seed of aril or aril hybrids. Since most arilbred seed are first year germinators there seems little point in trying to accelerate germination with them. Arilbred seed can be handled by plate culture or embryo culture, as well as natural planting.

In considering germination reflection indicates that we are not really too interested in rapid germination. The hybridizer needs substantial first year germination at such times as to assure maximum survival of the emergent seedlings. Seed from intercrosses of the C. G. White hybrids for example germinate very rapidly on fall planting (Wilkes 1962) in Southern California. For the mild climate of Southern California, and parts of Australia this is excellent. However, in the Mid-West, unless the seedlings are protected by hot beds, cold frames or greenhouses they often perish in the late fall and winter severe freezes. For Mid-West conditions spring germination is much better. Similarly embryo culturists have found that where the seedlings must be planted out in the open without shade protection that if they have not reached a certain stage of development the seedlings often go into a dormancy from which they do not recover. Oncos in the Mid-West on enlightened natural planting seem to germinate in mid-winter cold (Brizendine 1965). If they were to germinate in May it is extremely probable that their rhizomes would not develop to the extent that they could survive dormancy. Seeds quite generally have a preferred time of germination and this, of course, for species, is at a time which insures maximum survival. In the garden horticurists attempt to modify our plants inherited behavior to adapt it to survival under our localized garden conditions. The optimum time for germination of oncos can quite conceivably be different in Kansas or Illinois then in Southern California or Arizona.

If we go over the factors which aid germination one can relate them quite often to things happening to them in their native habitat. Inhibitors can be leached from seeds or at least some can. For aril species which are desert or steppe plants we see that this can happen in nature when the dried seed are leached in the fall and winter rains. After-ripening in some manner decreases or eliminates the effect of inhibitors. In nature we find the cold later fall and early winter periods subjecting the seed to after-ripening, and at other periods the seed is subjected to temperatures favorable to germination. A little reflection will convince us that seed in nature go thru a number of annual cycles, some seed germinating in the first year cycle and others in each following cycle until all have germinated which are germinable. There is a common belief that under dry storage with age that aril seeds become more easily germinated.

To develop a system of germination which will germinate our seed more or less naturally to a maximum extent at periods favorable to the survival of their seedlings it would seem natural to carefully consider these natural cycles. Crocker, Barton and co-workers at The Boyce-Thompson Institute have been highly successful in the development of germination methods for seeds which are germination problems with seed from many species and genera. Quite often the methods consist of shortening certain periods of the natural cycle such as after-ripening by conducting it at optimum temperatures and conditions, or in some instances by "compressing" a number of these annual cycles into a materially shorter period

It is in this last area that it appears that very substantial progress may be made. A maximum leaching can be most efficiently conducted by protracted soaking of disinfected seed with frequent changes of water, preferably daily. Provision for aeration of the soaking seed should be made by leaching with running tap water or by barely covering the seeds with water. During leaching seeds should be checked periodically to assure that they are not being contaminated by water molds or fungi, etc. While no really definite information exists as to how long seed may be soaked without harmful effects it appears it is substantial since 30 days is known to be safe. Warm water is perhaps more effective than cold for leaching. It is obvious that leaching operations as such are much more efficient in the laboratory than in nature. A solution of ordinary Sodium Hypochlorite bleach (i.e. Purex-Chlorox, etc.) diluted with an equal volume of water makes an excellent disinfecting solution for seeds. Seeds usually will be adequately disinfected by soaking in such solutions for a half hour. After disinfection the seed should be rinsed repeatedly with water until the chlorox odor is eliminated.

After-ripening is conducted by placing soaked seed in a damp porous material (so that the seed have access to oxygen of the air) and placing them in a cold place with temperatures between 30 and 40 Fahr. (the optimum temperatures for many species). The household refrigerator usually operates within this temperature range. All sorts of devices have been used for after-ripening — layering of seeds, stratification, etc., being alternate names. Plastic sandwich boxes with a cross placed between layers of damp spahgnum moss have been used for iris seed. Perforated to admit air they have the advantage that a relatively substantial group of crosses can be hand'ed at a time in a rather small space. Since germination sometimes occurs during after-ripening the seeds should be inspected at frequent intervals (i.e. weekly). Optimum periods for after-ripening of arils and aril hybrids are not known. Less than 90 days is usually ineffective while few species of seed require more than 180. From the natural cycle of onco growth and weather conditions in their habitats it is doubtful if conditions favorable for after-ripening exist over more than 4 - 5 months in nature. With the greater effectiveness of after-ripening under near optimum conditions it wou'd seem that 90 or 120 days would accomplish as much as can be done in a cycle.

One phase of aril lore for for which we have no clues in iris or comparable plants is that of the annual drouth period of dormancy. It is almost certain that during the summer months that the seeds dry to a degree at least and probably dry rather completely. Whether this drying out of the seeds in their natural cycle has any beneficial role is unknown. In some species of seed (not reported in iris) exposure of seeds to excessive temperatures is known to induce a so-called secondary dormancy. The stubborn dormancy of the oncos and some other arils (pseudo-regelias see Dykes reprint in 1954 Year Book) occasions speculation that such treatment may play a part in the germination problems we encounter.

Restated a germination cycle might be as follows: dehydration, leaching, after-ripening, exposure to germination temperatures (probably cold — Brizendine 1964), leaching (spring rains), and dehydration. For such cycles we cannot produce any evidence either way whether the dehydration portion of the cycle accomplishes anything. For crop seeds it is essential that seeds dry or "ripen" after harvesting. This is, however, based on economic reasons even as compelling as physiological. Crop seeds from generations of breeding have lost their inhibitors to a degree and it is necessary that they "ripen" or germination might occur at undesirable times. Stored in bins if

moisture is excessive grain heats with danger of killing the embryos in some cases to actual fire in others. These problems do not occur in ornamentals generally. No clear cut evidence exists that seed planted "out of the pod" produces superior or inferior plants to so-called "ripened" seed. Aril tradition from some of the pioneers is to the effect that planting right out of the pod hastened germination but no concrete evidence supports it to a too convincing degree.

If dehydration after harvesting and the normal germinating temperature exposure could be omitted successfully we have a 3-stage cycle. If we assume that the evidence of an optimum germinating temperature is in the 30-40 Fahr. range we may find germination occurring during our after-ripening cycle and thus have a two-stage cycle of leaching, with after-ripening and germination combined. Obviously several such cycles could be condensed into a year's time. If dehydration is an essential then perhaps we can compress only three such cycles in a roughly two year period. While some germination has been reported after 3 years for aril species it is doubtful if it is too significant, except in nature.

In considering dormancy one must stress the very great importance of first year germinating seed. Best present evidence is that 15-30% germination of aril seeds occurs in the first year. These seedlings are important since some will have genetic tendencies towards first year germination. The intercrossing of such can lead to the development of easy germinating strains, the seed of which can achieve reliable and substantial first year germination.

In reviewing some of our knowledge about germination no attempt is made to diminish the importance of embryo culture. In knowing hands it is our best and most certain method of handling difficult seeds. Even if we were to develop a method giving us spectacular germination results there will always be seeds with inadequate endosperm, no endosperm, or abnormal endosperm which cannot be germinated normally. Some of our most important seedlings come from types of crossings most apt to produce seeds of this nature. Workers intending to pursue such breeding lines should learn embryo culture. As has been stressed repeatedly in our literature the culturing of embryos is a rather simple procedure. The very general complication of embryo culture is the first transplant operation. In plant work it is often very difficult to duplicate the work of others. Using what seems to be identical methods some workers safely achieve in excess of 80% survival on first transplant, while others lose in excess of 80%. Slight variations in materials, water, humidity, temperature, etc., may account for such discrepancies. Hopefully we await a transplanting method which will work more generally everywhere with careful handling. The raising of aril seedlings reliably to first bloom is a necessity either with embryo culture or natural germination and one which individuals intending to work with arils must somehow master.

While references to more formal experimental work is useful, and many will wish to read the original papers referred to, this may be difficult for many readers. A very readable book on seeds, their production, dormancy, etc., is the 1961 Year Book of Agriculture entitled "Seeds". It will be available at most public libraries. Its low price of $2.00 may make it a desirable acquisition for your plant library. It may be obtained from the Superintendent of Public Documents, Washington, D. C.

As a matter of interest the annual handbooks of The Department of Agriculture quite frequently have material of interest to the gardener. Written in readily understood everyday language, and extremely readable they are

worth looking over at your local library. Each volume specializes on some particular phase of agriculture. While agriculture may seem a bit removed from horticulture we should remember that plants are to a degree plants whether raised by the few in gardens or by the acre on farms.

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EDITOR'S NOTE: Some confusion exists in the literature about the term "after-ripening". Many writers refer to "after-ripening" as synonymous with some of the well known cold treatments in which seeds are stored at low temperatures in contact with moist materials (sand, spahgnum, burlap etc.) for substantial periods of time or in controlled low temperature germinators. Mayer and Poljakoff-Mayber in their recent book on germination (1963) define "after-ripening" as dry storage after harvest, and use such terms as "layering" or "cold-treatment" to cover moist storage of seeds at low temperatures. Pollock and Toole (1961-U.S.D.A. Yearbook "Seeds") speak of the "drying and ripening" of seeds and thus use the normal nurseryman's ex-

pression for the *process going on* in seeds after harvest and in storage. They also speak of "after-ripening" in the dry condition. Nurserymen usually speak of *cold storage of seeds* in various moist media as "layering". The meaning of these terms usually is clear from the context, but in reading the literature *one should be careful* to be sure of the author's terminology. In books written by several authors this is especially true.