

Quality screening and evaluation in pulse breeding

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Abstract

Quality parameters of pulses (dry food legume seeds) are described. These include physical, physico-chemical and nutritional parameters. Chemical and nutritional composition of the major pulses are discussed, together with the relative heritability of some of the most important quality characteristics, and the advisability of attempting to breed for them. A strategy is outlined for the selection of pulses on the basis of quality factors which enables reduction of 10 000 early generation lines to three advanced lines in five selection steps.

Introduction

A pulse is defined here as a dry legume seed, intended for human or animal consumption. Pulses are distinct from oilseed legumes such as soyabean and groundnut.

There are four aspects of quality to consider in pulses or any other crop. These include: (1) Consumer acceptability in terms of food; (2) Consumer acceptability in terms of primary processing; (3) Nutritional value; and (4) Export specifications.

At the same time, there are five prerequisites for the establishment of a laboratory quality testing strategy for a pulse breeding programme. These are: (1) Identify the end-products in terms of foods or raw materials for which the pulses are intended; (2) Identify the seed characteristics which are responsible for consumer acceptance; (3) Establish the heritability of these characteristics; (4) Identify or develop laboratory test procedures which enable the characterization and evaluation of genetic material on the basis of quality; and (5) Verify that the test strategy developed realizes material that conforms to the quality aspects stipulated, by reference to standard samples.

Neither the aspects or prerequisites above are listed in order of importance; they are all of equal importance and largely interdependent.

Consumer acceptability depends mainly on appearance, price, texture, taste (including aftertaste) and nutritional characteristics. The appearance attracts people to buy or eat a food or raw material. It is strongly influenced by colour, but in terms of raw materials to the primary processor, seed size, seed uniformity (in size or colour) and freedom from damage from insects or fungi are all very important. The influence of price on consumer acceptability is fundamental and, in terms of certain basic staples, is sufficiently important to have strong political overtones. Colour is particularly important in lentil, where the deeper orange/red colour of decorticated, split, small-seeded (microsperma) lentil is often preferred. The degree of colour can vary from very pale to deep orange/red. The cotyledons of large-seeded (macrosperma) lentil, chickpea, pigeonpea, mungbean and black gram are usually yellow, the colour transmitted to the cooked foods. Some microsperma lentil also have yellow cotyledons, and these have their own specialized market. Colour, or rather shades of colour, does not appear to be a strongly-heritable characteristic. Texture of cooked foods is a function of cooking procedure as well as a seed characteristic. Taste in food legumes differs to a lesser degree between genotypes than texture. Other than obvious cases, such as extreme bitterness, the significance of any taste or aftertaste differences that do exist is questionable, in view of the herbs, condiments and other flavour substances (such as lemon juice) which are often added during cooking. Texture comes before taste — many taste components are released or at least amplified when food particles are bitten into. Foods which are too soft, too hard, sticky or slimy in biting texture are unattractive. This, together with inferior taste, causes rejection and wastage.

The nutritional characteristics of a pulse-based food present themselves to the consumer mainly in terms of anti-nutritional factors. These include digestibility or anti-digestibility, flatulence factors (gasogens), and toxic substances such as vicine and convicine, which are believed to be primarily responsible for a type of haemolytic anaemia known as favism. The nutritionist is concerned primarily with amino acid balance, protein "efficiency", digestibility and also with anti-nutritional factors, but it is the consumer who has to buy and process or eat the foods.

Consumer acceptance also involves industrial acceptability and export quality. A large proportion of pulses require primary processing before they can be processed into foods. This includes the milling of dhal from desi-type chickpea, mungbean, black gram and lentil, the decortication of lentil, the detexturization of kabuli-type chickpea, and canning of beans of both *Phaseolus* and *Vicia* species.

Genotypes with very small or very large seeds cause losses and other difficulties during cleaning, while seeds of some species, such as kabuli chickpea, that are too large are often very convoluted, which causes further trouble, due to the accumulation of dirt in the indentations. Seeds of species which require decortication and splitting, if they are very irregular in shape, also result in greater than average losses during decortication, which repre-

sents a financial loss to the processor. Determination of decortication loss should include assessment of the percentage of seeds which are split. Decorticated lentil and desi-type chickpea are usually marketed as splits, and the propensity of the seeds to both decorticate easily and split are important processing characteristics. The ICARDA-designed lentil decorticator (based on the F. H. Shule laboratory decorticator) is very effective both in decortication and splitting of lentil, and may also be useful in evaluation of desi-type chickpea, pigeonpea, green and black gram. Many seed characteristics are strongly heritable and can be identified by non-destructive methods (visual appraisal and sieving) in the early generations. For practical purposes, seed characteristics such as size, colour and shape are more important to the consumer than nutritional quality. By the F_4 – F_5 generations, all genotypes with undesirable seed characteristics can be eliminated by simple, rapid and non-destructive tests.

Surplus supplies of commodities, particularly of lentil and chickpea, are exported. Although the field pea is probably the most important pulse commodity involved in export trade, most of this trade is in Europe and other developed countries. The principal quality parameters for exporting include seed size, uniformity, shape, colour, freedom from external damage, freedom from foreign material, loss on decortication and moisture content, the latter being particularly important if a lengthy storage period is anticipated. With the exception of moisture and foreign material, which are not associated with breeding, all of these parameters are covered by normal screening for improved genotypes.

The release as cultivars of genotypes superior in yield and disease resistance, but inferior in primary processing, food or nutritional quality, is a retrograde and dangerous practice and reflects irresponsibility on the part of both the breeders and their institution. The increased wastage incurred by the reduced acceptability by both primary processor and consumer, together with the concomitant reduction in price are usually of greater magnitude than the statistically significant but practically insignificant increases in yield. Many quality parameters, such as seed size and shape, texture (including cooking time), amino acid balance and even protein content are generally more strongly heritable than yield and disease resistance, and can be improved fairly readily by breeding and selection, without sacrificing much in the way of yield potential. The much misused maxim "get the yield first and the quality can be added after" is a self-deception — breeders who have established a three percent increase in yield are more inclined to attempt to parlay this into an eventual six percent, rather than backcross in the essential quality parameters. In most cases where a cultivar combines satisfactory quality and yield, the basic quality parameters have arisen by accident rather than design, although there are doubtless some isolated instances to the contrary.

The principal foods and primary commodities derived from the major pulses are listed in Table 1 (the list is by no means exhaustive). A wide range

Table 1. Principal pulse-based commodities and foods.

Pulse	Species	Commodity	Food form
Dry bean ^a	<i>Phaseolus vulgaris</i>	Whole seeds	Boiled, stewed, fried, others
Dry pea ^{a,b}	<i>Pisum sativum</i> <i>P. arvense</i>	Split seeds	Boiled, soups, others
Faba bean ^b	<i>Vicia faba</i>	Whole seeds	Boiled, stewed, fried, others
Chickpea ^b (desi)	<i>Cicer arietinum</i>	Dhal, flour	Boiled, roasted, germinated, soups, fried, others
Chickpea ^b (kabuli)	<i>Cicer arietinum</i>	Whole seeds	Boiled, pureed, fried, roasted, detextured, others
Lentil	<i>Lens culinaris</i>	Split seeds, dhal	Boiled, steamed, soups, others
Mungbean	<i>Vigna radiata</i>	Dhal	As for chickpea (desi)
Black gram	<i>Vigna mungo</i>	Dhal	As for chickpea (desi)
Cowpea	<i>Vigna unguiculata</i>	Mainly whole seeds	Boiled, stewed, steamed, fried, others
Pigeonpea	<i>Cajanus cajan</i>	Dhal	As for chickpea (desi)

^a Also eaten as green, immature pods.

^b Also eaten as green seeds, raw or boiled.

of foods are prepared from most of the pulses, but most of them involve soaking, boiling or stewing, fermentation or roasting. Deep-frying of pulse flour "doughs" in various forms is also a popular method of preparation. Kabuli-type chickpea are often detexturized (made softer) and used in the preparation of snacks, such as qadami stamboulieh, qadami safra or qadami bisukar (sugared chickpea). Comprehensive cookbooks have been compiled to cover the recipes involving the faba bean, chickpea and lentil (Hawtin, 1979; 1981a, b). Methods of preparation of the cowpea have also been summarized (Dovlo *et al.*, 1976). Pulses such as chickpea, pigeonpea, cowpea, black and green gram are used after grinding into flours. Additional "flour" quality parameters such as foaming and emulsifying capacity and stability, fat and water absorption, all contribute to the usefulness and acceptability of a cultivar.

Nutritional value means the net contribution of a food to the metabolism of the individual which eats that food. All individual components of the food

contribute positively or negatively towards its efficiency. Some contribute to the essential amino acid balance, some contribute more to energy requirements, others contribute essential roughage, and the contributions of all individual components are affected by their net digestibility. If a food component is not readily digestible, it detracts from the contribution of other components, since extra energy is required to digest it. Similarly, the presence of anti-nutritional or toxic factors in a food can seriously detract from the overall nutritive value of that food.

Table 2 summarizes the proximate analysis of the major pulses; Table 3 lists the composition of limiting amino acids.

Food legumes are generally thought of as protein- and particularly lysine-supplements. Since they all contain starch, some sugars and variable amounts of oil, they also contribute significantly to the energy moiety of the diet. The

Table 2. Proximate composition (%) of food legume seeds.^a

Species	Protein	Starch + Sugars ^b (Total carbohydrate)	Crude oil	Crude fibre	Minerals
<i>Phaseolus</i> bean	22.1	61.4	1.7	4.2	3.8
Dry pea	22.5	62.1	1.8	5.5	2.6
Chickpea	20.1	61.5	4.5	4.9	2.9
Faba bean	23.4	60.2	2.0	7.8	3.4
Lentil	24.2	60.8	1.8	3.1	2.2
Pigeonpea	20.9	62.9	1.7	8.0	3.5
Cowpea	23.4	60.3	1.8	4.3	3.5
Green gram	23.9	60.4	1.3	4.2	3.4
Lima bean	19.7	64.8	1.1	4.4	3.4

^a From Aykroyd and Doughty (1964).

^b Determined by difference.

Table 3. Limiting amino acid composition of food legume seeds.^a

Species	Lysine	Methionine	Tryptophan	Threonine
<i>Phaseolus</i> bean	1.70	0.22	0.22	0.99
Dry pea	1.75	0.29	0.26	0.91
Chickpea	1.47	0.28	0.17	0.76
Faba bean	1.58	0.13	0.25	0.90
Lentil	1.57	0.20	0.20	0.91
Pigeonpea	1.54	0.24	0.10	0.82
Cowpea	1.67	0.47	0.15	0.85
Green gram	1.69	0.28	0.20	0.78
Black gram	1.81	0.35	0.12	0.91

^a Values are % of whole seed, based on mean literature values for protein ($N \times 6.25$).

three main nutritional drawbacks in food legumes are a serious deficiency in sulphur-containing amino acids (with the exception of the cowpea) and tryptophan, their variable and in some cases relatively poor digestibility, and their association with anti-nutritional factors. Generally, all pulses are deficient in methionine and cystine, and tryptophan may become limiting, especially in pigeonpea and black gram. The lysine concentration of chickpea is significantly less than most other pulses, and since its average protein concentration is also amongst the smallest of all pulses, some attention should be paid to the improvement of both parameters. A recent study by Singh *et al.* (1986) has indicated that the heritability of % protein of kabuli chickpea may be stronger than was originally believed. A second study by Robertson *et al.* (1986) has revealed that % protein in faba bean is also fairly strongly heritable. Furthermore, some studies have indicated only weak negative correlations between yield and % protein in food legumes (e.g. Griffiths and Lawes, 1978), so that it should be possible to improve % protein without sacrificing yield components. In view of these studies, it is possible that protein concentration of both pulse crops can be increased by breeding. Research should investigate the heritability of % protein in other pulses. Although there is a negative relation between lysine content of the protein and protein concentration in many crops, this is not sufficient to prevent an overall increase in lysine content of the seed when protein concentration is significantly increased. The same applies to other amino acids.

Protein quality can be described in two terms, amino acid balance and digestibility. The amino acid balance can be determined chemically (Spackman *et al.*, 1958) and the protein itself compared to a reference protein and expressed in terms of an amino acid score (Hulse *et al.*, 1977). Amino acid score is given by the content of limiting amino acid in the test protein divided by the content of the same amino acid in a reference protein (usually casein), expressed as a percentage. The most commonly-used method for evaluating protein quality biologically is the protein efficiency ratio (PER) method which involves comparing the nutritional effectiveness of the protein with that of a reference protein (casein), using rats as a test animal. The PER (Osborne *et al.*, 1919) is a single point assay, and is not linear across a range. For example, a PER of 2 does not mean that the protein is twice as good as a protein with a PER of unity. The net protein utilization (NPU) method of Bender and Miller (1953), and the more efficient net protein ratio (NPR) of Bender and Doell (1957), provide more comprehensive evaluations of proteins. Yet another method, the slope ratio or relative protein value (RPV), has been proposed (Said and Hegsted, 1970). This is based on the slopes of individual body weight response to protein concentration and, since it is a multidose method, it may become the method of choice, due to its apparently more comprehensive evaluation of a protein. The chief drawbacks of methods such as the RPV are their use of a relatively large amount of material and relatively long test periods, which limits their practicality to the

evaluation of advanced lines. All of these methods are strongly correlated, so the use of any particular method is essentially a question of convenience and preference by individual laboratories. For example, in comparing several diets by PER, NPR and RPV, the respective coefficients of correlation were PER:NPR = 0.985, PER:RPV = 0.986 and NPR:RPV = 0.994 (McLaughlan, 1977). Table 4 summarizes the nutritional values of selected pulses.

Table 4. Nutritional quality of selected pulses.

Pulse	Limiting amino acids		Amino acid score ^b	PER	NPU ^a
	Gross def'cy	Marginal def'cy			
Faba bean	Met., Cys.	Trypt.	28	—	48
Chickpea	" "	Trypt.	40	1.1–2.2	52–64
Dry bean	" "	Trypt., Val.	34	0–1.9	31–47
Dry pea	" "	Trypt.	37	0.3–2.2	41–50
Cowpea	" "	Iso-leuc.	41	—	35–51
Pigeonpea	" "	Trypt.	27	0.7–1.8	52

^a Bozzina and Silano, 1983.

^b A score of 100 indicates that the protein is equivalent to the reference protein in that particular amino acid.

Dashes for PER denote not available.

The heritability of PER, NPU and other protein evaluation methods depends on maintenance of amino acid proportions and digestibility from location to location and season to season, and must be established for individual crops before these tests can be of any value to a breeding programme. They are also time-consuming and only applicable to advanced material. Amino acid score is also a time-consuming operation, but uses less material. The advent of modern techniques such as near-infrared reflectance (Williams *et al.*, 1985) may simplify the screening of earlier generation material for limiting amino acids, so that genotypes with improved or very poor amino acid scores can be rapidly identified earlier in breeding programmes, provided the heritability of amino acid composition can be established.

Anti-nutritional factors include protease inhibitors, lectins, gasogens (flatulence factors), polyphenols (tannins) which are believed to affect digestibility, and toxic substances such as the favogens vicine and convicine. The most important aspects concerning these materials is to establish their concentrations in different genotypes, their heritability, and the extent to which they are destroyed or removed during primary or secondary food-processing. With regard to concentration, Pak and Barja (1974) found the dry bean (*Phaseolus vulgaris*) to have the most trypsin inhibitor activity, and

chickpea the least. The use of wild species to improve protein content and disease resistance may be tarnished by the greater concentrations of protease inhibitors in these wild species, recently reported by Singh and Eggum (1984).

Lectins, or haemagglutinins, are sensitive to heat treatment, and since most food legumes are boiled or steamed before serving, it is unlikely that these substances constitute a serious threat to nutritive value. Tannous *et al.* (1972) found both haemagglutinins and trypsin inhibitors to be practically destroyed in faba bean, chickpea, lentil and dry pea after 20 min autoclaving, while a trace of haemagglutinin activity remained in the dry (*Phaseolus*) bean. Gasogenic activity in pulses has been traced to the trisaccharide sugars stachyose, raffinose and verbascose. Chickpea is richer in these oligosaccharides than other pulses (Savitri and Desikachar, 1985). These substances persist after cooking and may even increase in activity in foods, presumably as a result of release during the cooking process. Again, the heritability of gasogen content has not been established. Polyphenols or tannins have been shown to inhibit the activity of digestive enzymes (Griffiths, 1980; Singh, 1984). These substances appear to be concentrated in the testa, and the removal of the seed coat improves biological values and net protein utilization (Hussein and Abbas, 1985). Seed coat colour can serve as an indicator of tannin content in some species and genotypes; lighter-coloured seed coats tend to be associated with less tannin in the *Phaseolus* bean (Elias *et al.*, 1973). This is of particular importance in the case of pulses which are not dehulled before cooking, such as *Phaseolus* and faba beans, and kabuli chickpea. Desi-type chickpea, mungbean, pigeonpea, black gram and lentil (microsperma types) are usually dehulled or decorticated before processing into food so that most of the tannins are removed.

Cooking quality includes cooking time and consumer acceptability of the cooked foods. A great deal of effort has been spent on studying cooking time and the factors affecting it in pulses (e.g. Halstead and Gfeller, 1964; Morris *et al.*, 1950; Quenzer *et al.*, 1978; Wassimi *et al.*, 1978; Youssef, 1978; Shrivastankar *et al.*, 1974). To some extent, this type of work has been inhibited by the lack of an objective method for the determination of cooking time. The best method still appears to be that of pressing the seeds with the fingers after boiling under reflux, followed by continued boiling and testing until a standard percentage (at least 50%, but preferably 90%) have become soft. Instruments such as the Instron and Ottawa texture testing devices give a less subjective evaluation, but in breeding programmes for pulses such as faba bean or kabuli chickpea, where cooking time of air-dry seeds can exceed 250 min, and seed availability is limited, they are of little value. The probes damage the seed coat, making the seed very permeable, so that they cannot be replaced into the boiling water. By using fingers as a probe, uncooked seeds can be replaced, so that sample size can be reduced. Cooking time is affected by permeability of the seed coat, by the texture or hardness of the seed cotyledon material, and by the physical size of the seed, which affects

surface area: volume ratio and the distance boiling water must penetrate in order to effect complete cooking. Of these, seed coat permeability and physical texture are probably the most important in most pulses.

Seed texture and seed coat permeability are both believed to be affected by the growing environment, where the temperature during the maturation phase appears to be important. The manner in which the seed components coalesce during maturation and its accompanying dehydration complement inherent genetic factors in establishing the final texture or hardness of the cotyledons, and also the composition and physical make-up of the seed coat. Faba bean and kabuli chickpea which mature under high temperatures appear to have longer cooking times than when the same genotypes mature under cooler conditions. This aspect of pulse cooking time requires further research. It is essential in quality work of all crops to consider the seeds as an entity rather than to attempt to identify single constituents that are responsible for differences in physico-chemical behaviour. Such parameters tend to be the result of interactions between several constituents and the environment. If a specific constituent is to be singled out, it is most likely to be plant and seed water status during maturation which exerts most influence on the texture and physico-chemical characteristics of seeds of all pulses (and cereals):

• In kabuli chickpea, strong correlations have been established between seed size and cooking time (Williams *et al.*, 1983; Singh *et al.*, 1986); significant relations have also been established between seed size and cooking time in faba bean and lentil (Singh *et al.*, 1987b). In practice, most pulses are soaked for several hours before cooking. This step significantly affects cooking time. The differences in cooking time between genotypes are greatly reduced, and in chickpea the relation between cooking time in dry and soaked seeds is not strong. These findings are summarized in Table 5.

• The significance of these findings to breeding programmes is that for all practical purposes, seed size alone is sufficient to enable the identification of genotypes with longer or shorter cooking time, but also, in view of the almost universal practice of pre-soaking, cooking time is of less significance as a quality parameter than was previously believed. Cooking quality evaluation

Table 5.(A). Influence of soaking on cooking time in three pulses.^a

Pulse	Mean SS (g 100 seeds ⁻¹)	Dry		Soaked	
		Mean CT (min)	SD	Mean CT (min)	SD
Faba bean large	143	274	52	76	10
Faba bean small	50	171	23	34	6
Chickpea large	48	148	29	46	10
Chickpea small	24	129	26	60	5
Lentil (microsperma)	4	32	7	9	3

Table 5.(B). Coefficients of correlation.

Pulse	SS:CT (Dry)	CT (Dry):CT (Soaked)
Faba bean (overall)	0.88	0.89
Chickpea (overall)	0.72	0.30
Lentil (<i>microsperma</i>)	0.91	0.85

^a SS = seed size; CT = cooking time; SD = standard deviation.

should include organoleptic testing of appropriate foods prepared by standardized procedures for respective pulses. Taste panels should include at least 15 members.

A strategy for quality evaluation in a breeding programme should employ as many non-destructive tests as possible in the early generations. Ideally, the test methods employed should enable the elimination of all inferior material, so that for later generations, provided that breeders pay heed to the advice of the quality laboratory, they should not need to concern themselves with quality — the more advanced (F_5 and onwards) material should all be satisfactory. The rigid rejection of 80 percent of the material (i.e. acceptance of the best 20%) will reduce a population of 10 000 lines to just three lines in only five steps. A suitable test strategy is suggested in Fig. 1.

Seed size can be determined by weight (usually of 100 seeds) or by sieves. Shape and colour are evaluated visually and are subjective in that they must both be evaluated by a grading system, established by the individual laboratory. Seed uniformity can be evaluated by means of sieves for most pulses. Dry (*Phaseolus*) bean and faba bean may be visually subdivided into large, medium and small seeds. Arbitrary values of 140, 90 and 50 g per 100 seeds can be set for large, intermediate and small faba bean. Protein can be determined with near-infrared reflectance (NIR) spectroscopy (Williams *et al.*, 1978), and individual amino acids by ion-exchange chromatography. Methionine can be determined more rapidly by the method of Mackenzie (1977), and recent work (Williams *et al.*, 1984; 1985) has indicated the possibility of using NIR spectroscopy for amino acid determination. This type of strategy enables the elimination of most of the undesirable genotypes on the basis of seed characteristics, while at the same time identifying lines with better protein and limiting amino acid contents. Screening for amino acids is suggested in early generations only if they can be determined with NIR. Otherwise, this testing would be deferred until after the fourth screening. Identification of protein-rich lines will, in most cases, automatically result in larger concentrations of all amino acids as a percentage of the seed. The early generation screening can be carried out on small amounts of seed, and the more time- and sample-demanding tests are deferred until the advanced generations when the number of lines is greatly reduced. This includes the determination of PER, RPV or any protein quality procedure

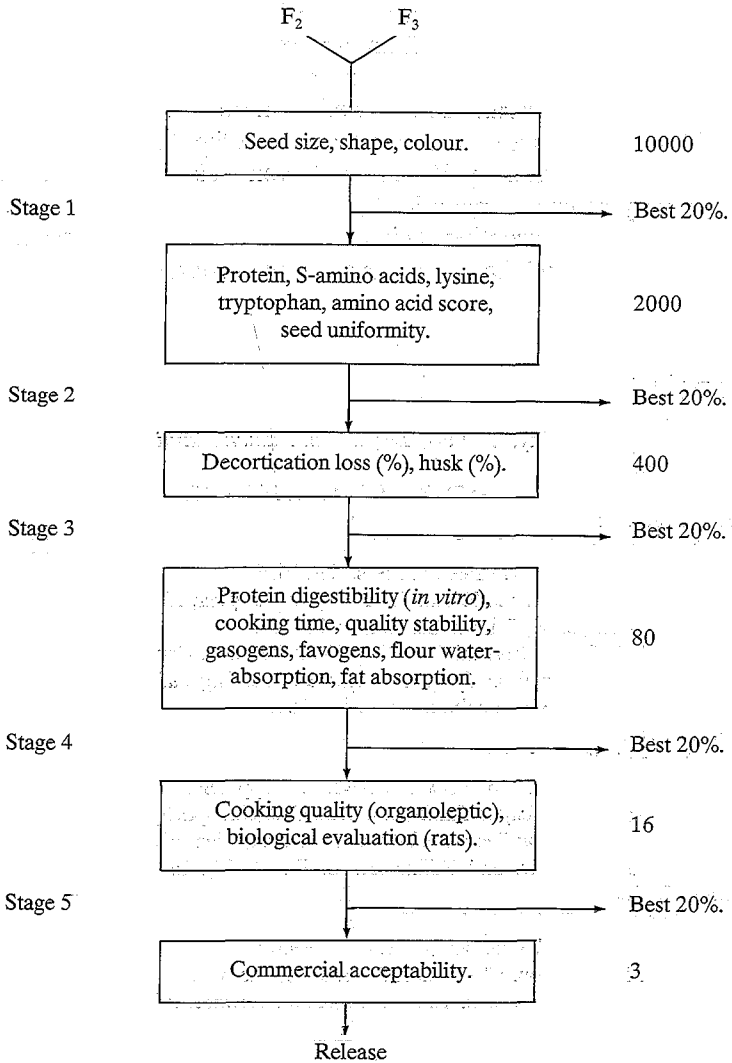


Fig. 1. Selection strategy for pulse quality screening.

involving animal studies. Organoleptic evaluation is also delayed until this stage.

Quality stability should begin at the third stage of screening, when the 80–100 lines left from a programme should be grown in at least two locations. At this stage, cooking time can be evaluated together with anti-nutritional factors. This strategy will achieve both the identification of “ideal” and unsuitable lines in individual locations, but will also realize the necessary information with regard to the heritability of these characteristics. Individual lines will also be identified which retain their compositional and processing

characteristics while others are far more variable. All of this information can be attained with a relatively small amount of testing. Elimination of most of the material on the basis of seed characteristics and other non-destructive testing in the early generations, followed by more comprehensive investigation of lines grown at more than one location at the third stage of screening, is the strategy preferred by ICARDA. Commercial acceptability is determined on very advanced material by providing retailers with several kilograms of seeds and accepting their advice as to their own acceptability as primary processors, and that of their customers as consumers.

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