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Predicting Tomato Seedling Morphology by X-ray Analysis of Seeds

W.J. van der Burg[1], J.W. Aartse, R.A van Zwol, H. Jalink, and R.J. Bino
Centre for Plant Breeding and Reproduction Research, CPRO -DLO, P.O. Box 16, Wageningen NL-6700 AA, The Netherlands

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***Abstract.* Studies based on X-ray photographs were conducted to predict the morphology of tomato (*Lycopersicon esculentum* Mill.) seedlings at transplanting stage. Currently, seed-lot quality of tomato seeds for growing commercial transplants is determined with grow-out tests in the greenhouse because the standard germination test fails to predict the percentage of normal or usable transplants (UTs). These grow-out tests, however, are difficult to standardize. An X-ray evaluation procedure is presented as an alternative. X-ray images nondestructively provide information on embryo size and morphology and the amount of endosperm and the area of free space. These parameters correlate well with the morphology of 14-day old seedlings. Cotyledon morphology has the highest correlation with the percentage of UTs. A test based on the evaluation of X-ray images, classifying the cotyledon morphology and seed free space, predicts the percentage of UTs more accurately than the currently used germination test. A second method based on an equation that uses the probabilities of all X-ray categories proportionally predicts the percentage of UTs of primed seeds more accurately than the first method. Selecting individual seeds based on X-ray images has the potential to raise the percentage of UTs of seed lots. On the average, the percentage of UTs of control seeds was 22% higher after hand selection based on X-ray evaluation. Primed seeds gave 12% higher results. Hence, X-ray analysis can predict seedling performance and enable the selection of high-quality seeds.**

The germination tests performed according to the rules of the International Seed Testing Association (ISTA, 1993) or the American Association of Official Seed Analysts (AOSA, 1989) estimate the percentage of seeds capable of germinating normally. These tests are performed under ideal conditions for seed germination and are based on a morphological evaluation of seedling characteristics. Cotyledon and root morphology are analyzed. However, seedlings with large defects are still considered normal if ≥50% of the cotyledonary surface is effective, because such cotyledons can support the normal growth of a seedling. Hence, small abnormalities are not considered in the standard germination test.

The current practice of tomato cultivation in most European and North American countries includes the production of seedlings by professional growers. The quality of the transplants is determined by the morphology of the cotyledons and first leaves and by the uniformity in development. The root system is not evaluated. The criteria used on the above-ground parts are often very strict. Consequently, the standard germination test does not produce the data necessary for this particular facet of trade. As a result, seed lots are currently being rejected for transplant production on morphological grounds, despite good performance in the germination test (generally >90% germination). Currently, the germination test has been replaced by a grow-out test in Holland. Evaluation takes place 10 to 14 days after sowing, and the percentage of saleable transplants is referred to as the percentage of usable transplants (UTs). In this study, we have adopted this terminology. Seeds are traded and production is planned on the basis of these tests. Standardizing greenhouse experiments is difficult due to variation in potting media and seasonal effects. Consequently, these greenhouse trials are subject to large variations.

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[1]To whom reprint requests should be addressed.

There is a great need for seed lots that produce high percentages of UTs and require less manual handling to reduce the risk of spreading diseases and reducing production costs.

X-ray analysis of internal seed structures provides a technique to evaluate seed quality (Chavagnat and Le Lezec, 1984; Simak, 1991; Swaminathan and Kamra, 1961). The analysis is nondestructive and allows the morphological evaluation of the embryo and endosperm in the intact seed. Using X-ray analysis, Argerich and Bradford (1989) reported the occurrence of free space between embryo and endosperm in primed tomato seed after drying compared to control seeds.

Our objective was to develop the potential of X-ray analysis for the rapid evaluation of the internal structures of tomato seeds and their correlation with seedling morphology. We describe using X-ray images of dry tomato seeds to predict the percentage of UTs based on the area of free space and embryo morphology.

Materials and Methods

Plant material. Five seed lots representing three cultivars of tomato hybrids were obtained from commercial sources (Table 1). From each lot, 200 control (nonprimed) seeds and 200 primed seeds were used. Priming occurred in darkness at 20C in –1.0 MPa polyethylene glycol (PEG) 6000 for 7 days. The seeds were dried back at 20C and 32% relative humidity for 72 h.

Seed germination. The grow-out test for tomato seedlings was performed in an air-conditioned greenhouse in standard potting soil in June 1991 without additional artificial lighting. The maximum temperature was controlled at 27C.

Seeds were germinated in the laboratory on top of moist filter paper with additional lighting at alternating temperatures of 20 to 30C (ISTA, 1993), and held in a germination cabinet in covered boxes. All seeds were X-ray photographed and 100 seeds per treatment were individually evaluated with X-ray analysis before being sown in the greenhouse.

X-ray photography. X-ray photographs were prepared with a Hewlett-Packard Faxitron 43805N X-ray apparatus using Agfa

Structurix D4 X-ray film exposed at 10 kev, 2 mA (soft X-rays) at a distance of 35 cm from the X-ray source for 5 min. Seeds were placed in a cassette-type holder, 50 at a time, each in a separate place. The seeds were in direct contact with the film during the X-ray exposure. The film was developed in Agfa Neutol paper developer and evaluated using a standard library microfiche reader (Minox 33200).

Photograph analysis. X-ray photographs were evaluated based on embryo morphology and the presence of endosperm. Embryo morphology was scored as either normal or abnormal (Table 2). A normal embryo contained two perfect cotyledons that were curled in the plane of the photograph (Fig. 1). Two basic configurations can be observed in tomato seeds: coiled (Fig. 1 a and b), where both cotyledons are rolled in together, and spectacles (Fig. 1 c and d), where the cotyledons are coiled in opposite directions. The abnormalities found in the embryo were mainly observed in the cotyledons: strongly folded, wrinkled, broken or otherwise deformed. Hence, an embryo was considered abnormal if one or both cotyledons were curved sideways (Fig. 2) or deformed. Root abnormalities were rarely observed. The roots were either plump and solid or more acute and slender. The latter was only observed in seeds with free space (Fig. 1 b–d). Both types were considered normal, but a few roots showing clear signs of degradation were considered abnormal. The presence of endosperm or, conversely, the area of free space was scored using three classes: none = no free space (endosperm completely filling the seed); little = free space no more than 50% of the embryo size; and extensive = more than 50% of the size of the embryo as free space.

The above provided 2 × 3 X-ray categories—categories 1 to 6 (Table 2). In addition, category 7 comprised seeds without an embryo or completely empty seeds, and category 8 comprised seeds that could not be categorized (insufficient details in the photograph).

Table 1. Seed lots used for the experiments.

Lot	Cultivar	Germination (%)	1000-Seed wt (g)	Producer
A	Lerica	97	2.27	Zaadunie, Netherlands
B	415	97	2.54	Bruinsma Seeds, Netherlands
C	Carmello	97	4.00	Zaadunie, Netherlands
D	Carmello	80	4.03	Zaadunie, Netherlands
E	Carmello	91	3.49	Zaadunie, Netherlands

Table 2. X-ray evaluation categories based on embryo morphology and area of free space.

X-ray category[z]	Seed evaluation criteria: Embryo morphology	Seed evaluation criteria: Area of free space[y]
1	Normal	None
2	Abnormal	None
3	Normal	Little
4	Abnormal	Little
5	Normal	Extensive
6	Abnormal	Extensive
7	No embryo	No endosperm
8	Classification not possible	

[z]Category numbers as used for final scoring of the seeds (see text).
[y]The area of free space was scored using three classes: none = no free space (endosperm completely filling the seed); little = free space ≤50% of embryo size; and extensive = free space >50% of the size of the embryo (see Figs. 1 and 2).

Seedling morphology prediction. Two methods for assessing internal seed structures as predictors for seedling morphology were used.

Method 1 is based on the rationale that only normal embryos would produce normal seedlings, and that the area of free space should be limited. The assumption was that X-ray categories 1 and 3 (Table 2) would together represent the seeds capable of producing UTs.

In Method 2, a synthesis of the predictive values of the individual categories was used) to improve the prediction. Most categories will produce at least some UTs, although with different probabilities. The equation used in Method 2 consisted of summing all observed frequencies and multiplying with their probability factor: predicted percentage usable transplants = $\sum f_i p_i / \sum f_i$, where p_i is the probability of usable transplants and f_i is the number of seeds for category i (i = 1...8). The probability p_i was calculated by dividing the number of seeds producing UTs by the number of seeds observed per category (Table 3), while excluding the lot that was to be predicted. Thus, the prediction of UTs of a given lot was determined with a probability factor p_i calculated from the other nine lots.

Seedling analysis. The seedlings grown in the greenhouse were scored as follows: category A, seedlings without any visible defects (Fig. 3a); category B, seedlings with only very slight morphological defects in the cotyledons (Fig. 3b); and category C, seedlings with slightly more prominent to severe defects (Fig. 3 c and d) or no germination at all. Delayed seedlings (Fig. 3e) were also included in category C, because this retardation resulted in nonuniform batches of seedlings. Categories A and B were considered to be UTs, and together made up the percentage of UTs. The seedlings grown in the laboratory were evaluated following the descriptions of Bekendam and Grob (1979).

Results and Discussion

Seed structure evaluation. The results of the germination test and the percentage of UTs are given in Table 4 along with predictions of UTs based on internal seed morphology. The seed lot with the lowest germination capacity (D) also provided the poorest percentage of UTs (Table 4). Only about half of the seeds of this lot capable of germinating normally in the standard germination test was able to provide UTs. This difference was also substantial in some (e.g., C and E) of the four lots with much greater normal germination (Table 4). The discrepancy between the germination results and percentage of UTs (Table 4) is mainly due to the fact that the criteria for normal seedlings in the standard germination test are less strict than those for evaluating seedlings for the percentage of UTs.

The degree of success with which X-ray analysis enables seedling morphology to be deduced from internal seed structures depends on the species (Simak, 1991). In mature tomato seeds, the embryo is fully developed and, because the seed is laterally flattened, the resulting X-ray photograph provides a clear side view of the embryo. All internal structures, such as the primary root, the hypocotyl, the two cotyledons, and the endosperm, are readily visible (Fig. 1). The primordium of the shoot (plumule) is not yet visible at this stage. In most tomato seeds, the endosperm surrounds the embryo and fills the remainder of the seed, but some seeds manifest so-called free space between the embryo and the endosperm (Argerich and Bradford, 1989; Fig. 1 b–d). In all cultivars and seed lots studied, primed seeds showed free space more often than control seeds (99% and 31% of the seeds, respec-

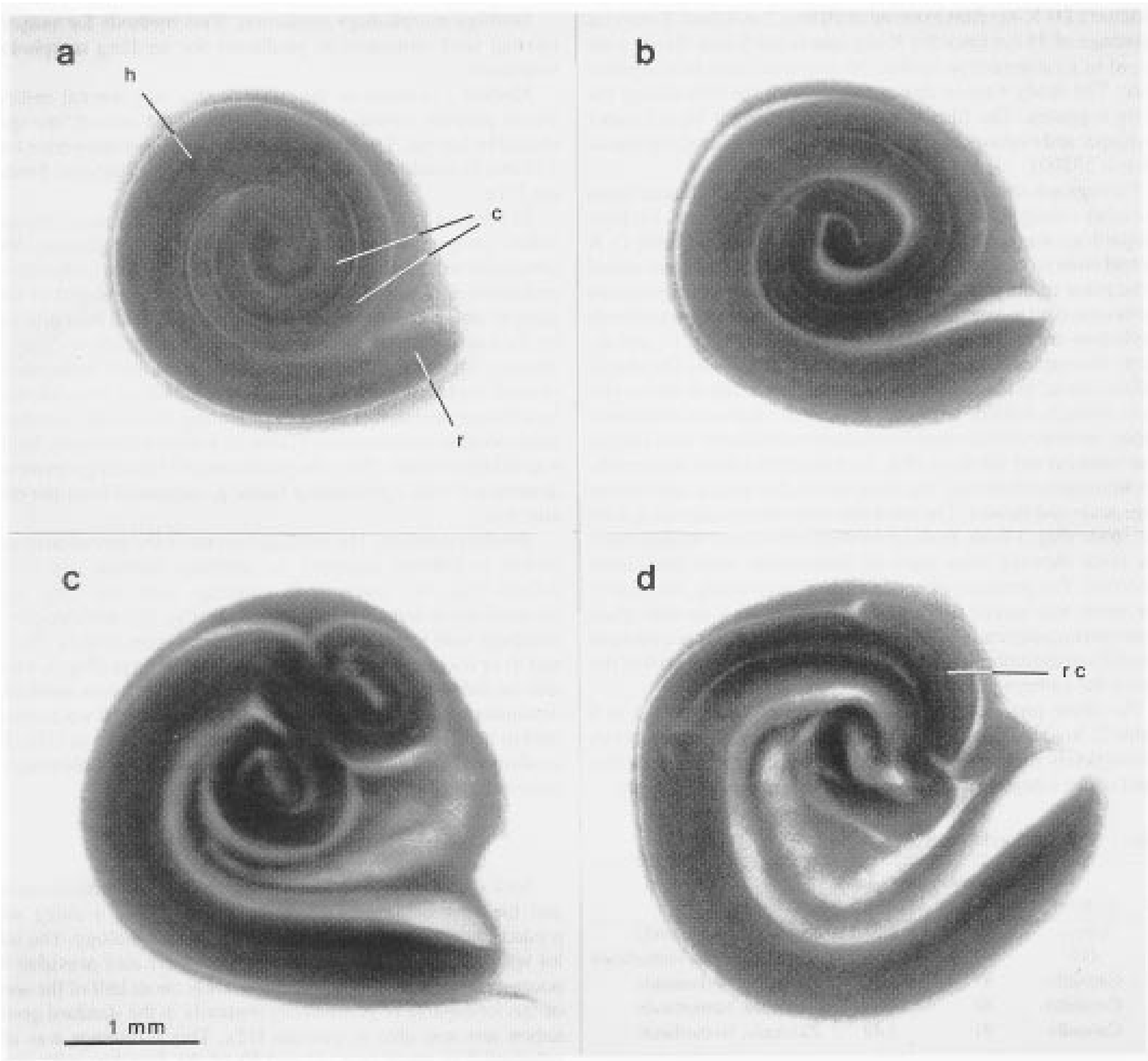

Fig. 1. X-ray photographs of tomato seeds showing the two basic cotyledon configurations, morphology classes, and amounts of free space. Seeds **a** and **b** represent the 'coiled' embryo configuration. Seeds **c** and **d** represent the 'spectacles' configuration. Seed **a** is of class none (no free space, endosperm completely filling the seed), seeds **b** and **c** are of class little (free space is ≤50% of the embryo size; free space appears as white areas in the seeds), seed **d** is of class extensive (free space >50% of the size of the embryo). In seed **d**, the outer cotyledon is sharply reflexed (indicated by the bar); h = hypocotyl; c = cotyledons; r = root tip; rc = reflexed cotyledon.

tively). The occurrence of free space makes the embryo more clearly visible and facilitates X-ray evaluation. Argerich and Bradford (1989) observed the embryo clearly in 60% of primed and 20% of control seeds. In our studies, 100% of primed and 95% of the control seeds could be evaluated. This difference in results may be due to a combination of a better photographic technique and the experience gained with the large numbers of tomato seeds studied. Also, some tomato cultivars may give clearer pictures than others.

Internal seed structures and transplant morphology. Shedding the seed coat is an important factor in normal seedling development. If the cotyledons remain (temporarily or permanently) within the seed coat, they become subject to various degrees of damage. Usually the cotyledon edges show nicks or indents (Fig. 3 b–d).

The successful shedding of the seed coat depends on various factors. Internally, the embryo must be intact and have one of two ideal configurations: either coiled (Fig. 1 a and b) or spectacles (Fig. 1 c and d). The coiled embryo seems to be the basic configuration in tomato and related species, like pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.), in which we never observed the spectacles type. The latter, although it would seem less efficient, gave equally well developed seedlings as the coiled type. However, in either case, one or both cotyledons may be sharply reflexed inside the seed (Fig. 1d). Seeds that contain cotyledons with larger sharply reflexed tips generally develop into seedlings with abnormal cotyledons. The reflexed tips may break off or cause scars at the cotyledon edges. The seed coats are then often shed at the cost of the tips of cotyledons (Fig. 3e): the tips break off and the cotyledons unfold without them.

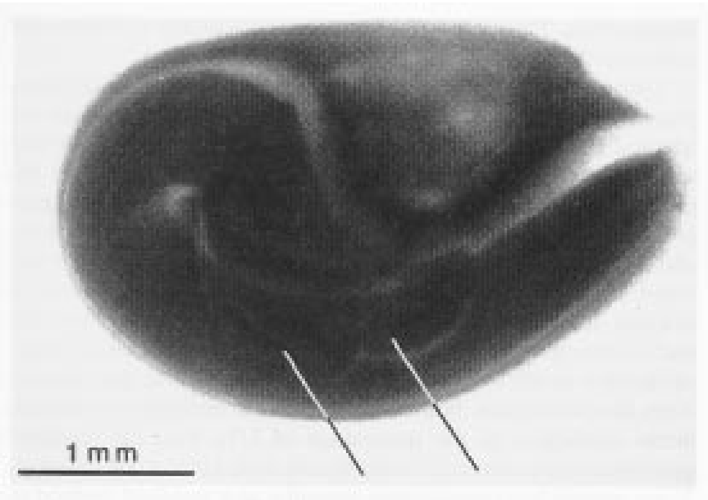

Fig. 2. X-ray photograph of a seed with the cotyledons (indicated by bars) curved sideways, i.e., out of the plane of projection.

The amount of endosperm is also critical for normal seedling development. Categories 5 and 6, which have extensive free space, rarely gave UTs. However, even if all morphological traits are optimal, the environment can still cause considerable variation in seedling development. This variation in the behavior of seedlings under different conditions makes it difficult to develop a seedling-based test that is independent of environment. The advantage of X-ray analysis is that it is independent of such external factors and, therefore, the test is likely to be more reproducible.

Table 3. The expected percentage of usable transplants (UTs) per X-ray category over all seed lots.

	Control seeds		Primed seeds	
X-ray category	No. of seeds	Expected percentage of UTs (p_i)	No. of seeds	Expected percentage of UTs (p_i)
1	279	89	7*	57
2	65	28	0*	---
3	38	79	375	90
4	48	38	107	43
5	6*	67	4*	0
6	38	24	6*	17
7	1*	0	0*	---
8	25	20	1*	100

*Statistically insufficient numbers were available for a reliable estimate of p_i.

Using Method 1, which consists of the classification of X-ray categories 1 and 3 as potential UTs, this prediction was close to the percentage of UTs observed in the greenhouse (Table 4). Moreover, this method gave a much better prediction of percentage of UTs than the standard germination test; the latter deviated significantly from observed UTs.

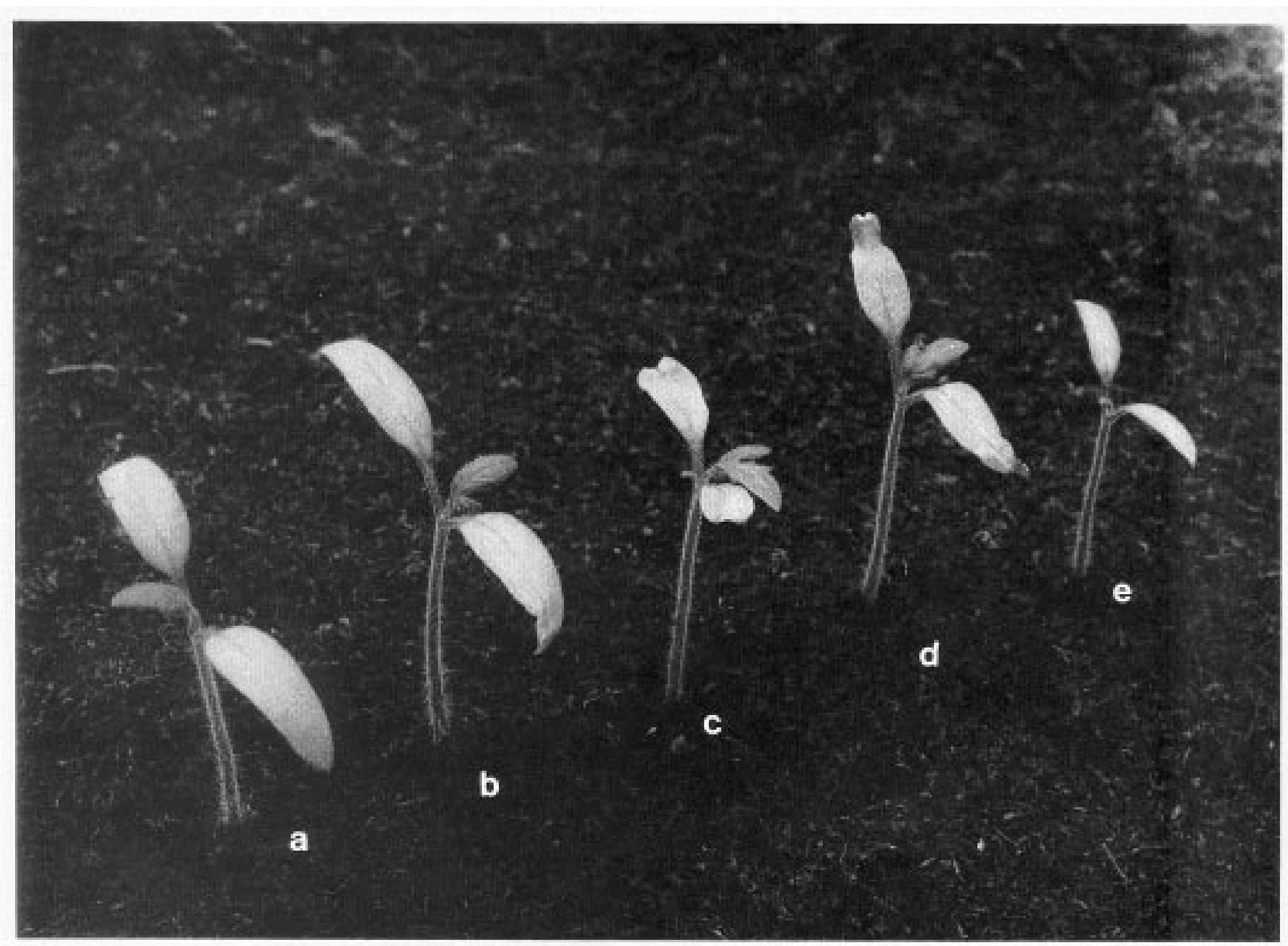

Fig. 3. Types of seedling abnormalities: (**a**) undamaged category A seedling; (**b**) category B seedling with only slight defects; (**c** and **d**) category C seedlings with defects of such severity that they were not considered usable transplants; (**e**) a normal seedling that is too small.

Table 4. Usable transplant (UT) percentage of tomato seeds predicted by two X-ray evaluation methods compared to the standard germination test (percentages).

Lot	UTs[z]	Germination capacity[y]	Prediction of UTs Method 1[x]	Method 2[w]
A control	93	97	92	79
A primed	89	97	95	86
B control	93	97	82	72
B primed	85	99	92	84
C control	55	96	51	60
C primed	67	97	52	64
D control	36	80	38	61
D primed	70	86	67	73
E control	54	91	54	61
E primed	79	93	76	77
Mean	72	93	70	72
Mean of paired differences from percentage of UTs				
Control	---	26.0	3.6	18.4
Primed	---	16.4	6.8	2.4
Total	---	21.2	5.2	10.4
SD	---	15.4	6.8	12.3

[z]Actual percentage of UTs observed in the greenhouse.
[y]Percentage normal seedlings according to the procedures of the International Seed Testing Association (1993).
[x]Percentage of UTs predicted by visual analysis of X-ray photographs using Method 1, where percentage UT equals percentage of seeds in categories 1 and 3.
[w]Percentage UTs predicted by Method 2, in which the equation presented in the text accounts for the probability of all fractions.

The mean of unsigned paired differences (difference between the predicted value and actual percentage of UTs) is 5.2 for the whole series. This figure was better for control lots (3.6) than for primed lots (6.8).

Using Method 2, which uses the probabilities of all categories proportionally, the overall prediction was less accurate than for Method 1 (Table 4): the mean of paired differences for the whole series was 18.4. The method performed much better with the primed lots (mean of paired differences 2.4), a result indicating its potential for this category of seeds. The method depends on exact classification of the X-ray images. Therefore, Method 2 performed better with primed seeds, which usually show more prominent features. In future studies, the factor p_i will be further developed for each X-ray category and its dependence on factors such as cultivar, production conditions, and period of sowing will be determined. Notwithstanding that, both X-ray evaluation methods give a much better prediction of the percentage of UTs than the standard germination test. Hence, it may be concluded that a test based on X-ray images has considerable potential as a standard method to predict percentage of UTs.

Sorting. Apart from using X-ray images to predict percentage of UTs, they may also be used to select high-quality seeds with high rates of UTs. The effect of a possible sorting based on X-ray analysis was calculated from the X-ray results and Method 1. The percentage of UTs before and after artificial sorting are presented in Fig. 4. The artificial sorting consisted of selecting seeds of categories 1 and 3 and determining their percentage of UTs. The average percentage of UTs of nonprimed control lots increased from 66% to 88%, and from 78% to 90% for primed lots. In individual cases, especially with low-quality lots, the results may

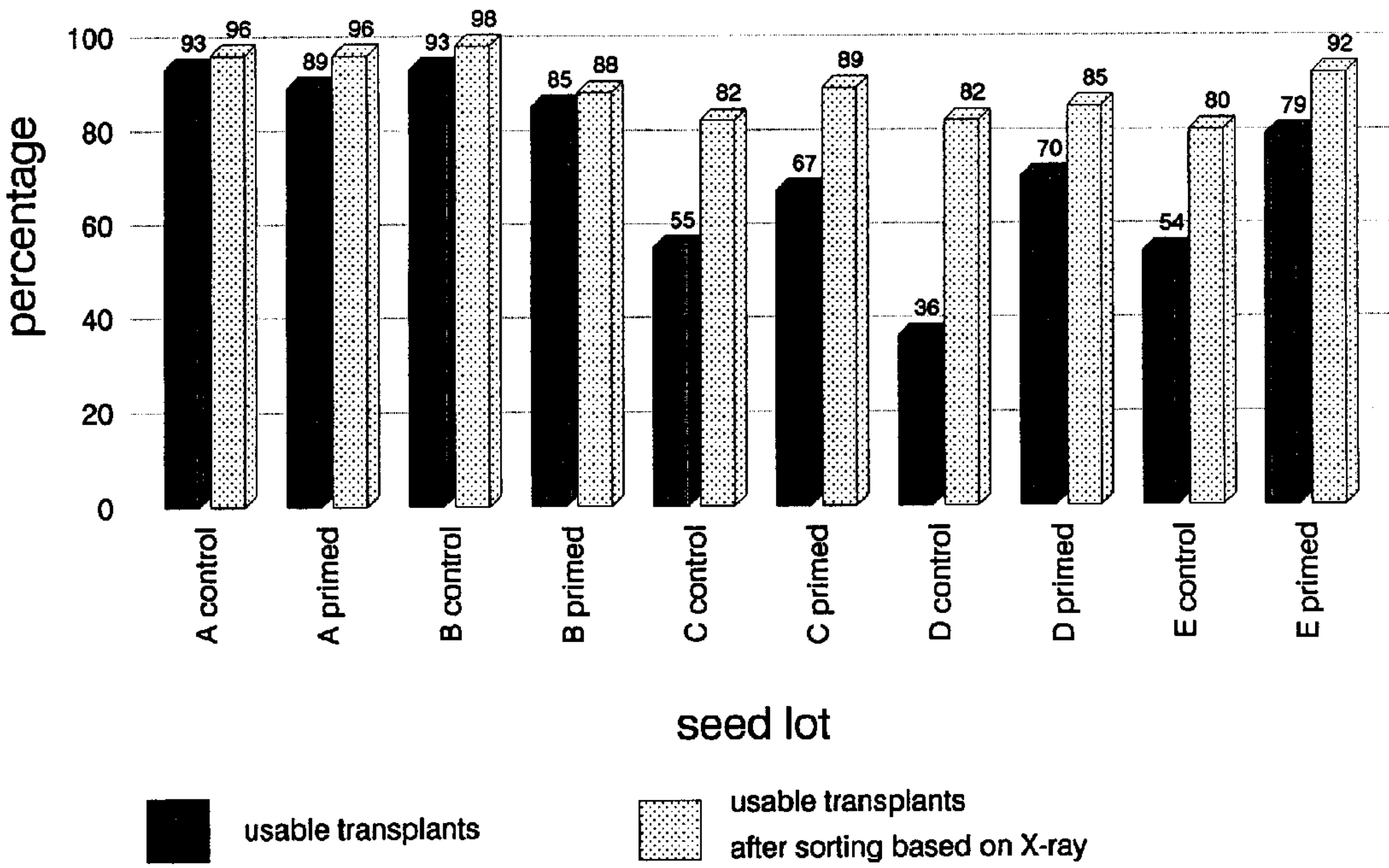

Fig. 4. Improvement of seed quality, expressed as usable transplant percentage, as a result of selection based on visual evaluation of X-ray images.

be even more prominent. In theory, 100% UTs would be achievable, but, due to natural variation, some seeds that show good characteristics in the X-ray test fail in the grow-out tests. Possible reasons are local unfavorable conditions for some seeds to germinate, invisible infections with micro-organisms, and physiologically dead seeds (e.g., due to overheating, overtreatment, age).

Notwithstanding that, these quality improvements are substantial and are of direct relevance to the seed trade. Currently, however, results are based on visual examination of the photographs, which is time-consuming and requires considerable expertise. A method for the on-line capture of X-ray images with high resolution and speed is currently under investigation.

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