Asian Journal of Plant Sciences

ISSN 1682-3974

Asian Journal of Plant Sciences 5 (3): 492-497, 2006
ISSN 1682-3974

Microstructure of Dormant Cotton Seeds

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Abstract: The roentgenograms of so-called stony, non-swelling, hard seeds of *Gossypium hirsutum* L., *G. barbadense* L. and *G. herbaceum* L. have been investigated. Abnormal morphological structures have been found without germination test. The original data on micro hardness of cotton seed peel and its structural layers are given. The obtained data form new approaches to a problem of seed hardness.

Key words: *Gossypium*, seed, stony seed, contact radiography, seed dormancy, micro hardness, light line of peel of seeds

INTRODUCTION

Hard or stony seeds with labored germination and dormancy are found for the majority of the plants with hard seed coat in particular, for *Malvaceae* family (Fryxell, 1969). *Gossypium* L. belongs to this family and it includes about 70 cotton varieties (Dariev and Abdullaev, 1985). Five of them are used for cultivation.

The problem of seed hardness is of biological and practical importance; many works are devoted to it (Walhood, 1956; Christiansen *et al.*, 1960; Konstantinov and Volkova, 1960; Curtis, 1963; Yatsu, 1965; Wareing, 1971; Poptsov, 1976). It was observed that seed hardness is strongly dependent on moisture and temperature of the environment during seed maturation, as well as mechanical damage of seeds, micro flora, environmental gas composition, physical and chemical action of the seeds for seed hardness elimination, inorganic nutrition of plants, storage and structural features of the seed coat (Dariev and Abdullaev, 1985). Many researchers found that the seed hardness is related to the waterproofness and gasproofness seed coat, especially to its palisade, columnar layer.

For example, for cotton seeds, prismatic, thick-walled cells of this layer in the top third part of its length very densely adjoin each other. When the cuts of the seed coat are examined, an original optical effect in the form of brightly luminous line is seen, which is called light line (Philimonov, 1961). The light line was found in all families, whose representatives have hard seeds (Krakhmalev and Sultanova, 1983).

It is known that among cotton cultivars the hardness of seeds is seldom observed (2-6%) (Konstantinov and Volkova, 1960). However, in the cotton population there are the lines with mainly hard seeds (Poptsov, 1976) and their behavior is identical to the behavior of hard seeds of arboraceous-shrubby species of leguminous. Many of hard, stony seeds are found among natural cotton species (Fryxell, 1969; Dariev and Abdullaev, 1985; Konstantinov and Volkova, 1960; Christiansen *et al.*, 1960).

The tests for germination ability at 24°C were carried out on the seeds of three cotton species (*Gossypium hirsutum*, *G. barbadense*, *G. thurberi*) (Walhood, 1956). It has been shown by Walhood (1956) that for *G. hirsutum* L. and *G. barbadense* L. the amount of hard seeds, ungerminated after five day in laboratory tests, was within the limits of 4-44%. *G. thurberi* cotton seeds did not germinate at all during this period (Walhood, 1956). They germinated only after thermal processing in hot and cold water after the fifth day by 82 and by 84% after drying and storage for two months. The rest non-germinating seeds 18% of the investigated sample were absolutely hard.

Seed hardness, which is characteristic for natural cotton species, is based on dominant genes carrying extremely significant attributes, which are absent for the cultivated species. They have a reduced susceptibility to verticilliose and fusarial wilt, frost-resistance, resistance to many diseases of bacterial character, etc. (Dariev and Abdullaev, 1985). As it is known, hybridization of natural species gives wide possibilities for genetic variability and allows obtaining species with advanced properties. However, the inheritance of seed hardness also can take place. It has been found out, that one can predetermine the presence of such stony seeds in seed grain with sufficient accuracy without the use of standard methods of cotton seed germination under laboratory conditions. The radiographic investigations show that it is related to

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the features of morphostructure of the embryo. The X-ray test has been considered by many authors of potential value because it is accurate, quick and nondestructive. In this context abnormalities in aroeira-branca embryos by radiography method were investigated by Macheeb and Cícero (2003). Seeds with an embryo/embryo cavity proportion lower than 100%, as determined by the X-ray test, did not germinate and those with abnormal embryos produced abnormal seedlings or did not germinate, providing evidence that the X-ray test is effective to assess seed quality. Investigations of seed morphology and development, seed collection and sorting and seed viability were conducted in species of Cannomois Desv., Hypodiscus Nees and Willdenowia Thunb. (Newton *et al*., 2002). Suitability of collection methods and the effectiveness of sorting techniques were evaluated using x-radiography. Viability was generally high (mostly>80%), suggesting that poor germination in studies with well-sorted seeds is likely to be due to seed dormancy (Newton *et al*., 2002). According to Rosok and Meland (2004) anatomically developed seeds can be physiological immature and will not germinate. X-ray pictures of immature seeds with high water content are greyish with low contrast and difficult to classify correctly and as pointed out by these authors, some types of seed damage are invisible to X-rays such as damages on root and seed-coat, fungus infections, frost damages and abnormal embryos.

As we can see, dormancy in cotton seeds is connected with morphological features of the embryo and seed coat. To observe the embryo microstructure, we use roentgengraphy images on photo film by using soft X-Rays (5-10 KeV). To get more fine details of the embryo morphostructure, an optical microscope (Neophot-2, Carl Zeis Jena) was used. Combination of these two methods generates a full detail picture of cotton seed embryo of dormant seeds. Alternativey, the reason of dormancy may be connected to the high hardness of the seed coat. Therefore it is necessary to discuss the influence of microhardness of the seed coat on its germination. The objective of this research is, thus, to determine morphological features of dormant cotton seed embryos and seed coats by a combination of X-Ray image, optical microscopy and microhardness determination methods on seed coats of *Gossypium hirsutum* L., *G. barbadence* L. and *G. herbaceum* L.

MATERIALS AND METHODS

Mature seeds of following cotton varieties were investigated: Tashkent-1, 108-F, C-4727 (*Gossypium hirsutum* L.), C-6030, C-6524 (*G. barbadense* L.), widely cultivated in the Republic of Uzbekistan and also coarse-fibered form *Turfan Guza* (*G. harbaceum* L.) collected in 2005 at experimental plot of Institute of Electronics Uzbek Academy of Science. After hand ginning the seeds with dark brown and brown coloration in a quantity not less than 1000 seeds were placed into sandy germinators and couched under laboratory conditions in thermostats during 10 day according to a procedure established by Rakhimov and Rudenko (1976). Non-germinated seeds with undamaged seed coat were cleaned from the rests of sand and were dried at room temperature; then they were set on special holder in the Electronics-25 device for radiographic analysis. Special frames allowed to place cotton seeds directly on a negative film with the size of 13×18 cm. After exposure to soft X-Rays the films were developed and explain roentgenograms of stony seeds were obtained. The operating modes were the following: tube voltage -18 keV, tube current -40 mkA, exposure time-2 min (Krakhmalev and Alimova, 1981; Krakhmalev, 1991). Contact roentgenograms of the seeds were placed into photographic enlarger, giving on photographic paper the increased patterns of fine structure of stony seed embrio, available for observation. It is necessary to note that in addition to stony seeds, the empty seeds also did not germinate during laboratory tests and the rest of endosperm was seen inside them. In the given case the use of radiography is caused by its high resolving power and by the possibility to study the seed germs without its breaking (Smirnova, 1978).

RESULTS AND DISCUSSION

When the hairs removed, mature cotton seeds have dark or dark brown color of seed coat and high microhardness almost of all constituent structural layers (Krakhmalev and Gershman, 1990). For example, if in process of maturing, the microhardness of external epidermis of seeds for 108-Φ *G. hirsutum* L. cotton sort changes within the values of 18-28 kg mm^{-2}, the microhardness columnar (palisade) layer (at a level of middle of cell cavities) increases from 20 to 30 kg mm^{-2}. The greatest jump of microhardness during maturation is observed in a thin interlayer of seed coat, passing through top ends of columnar cells, where under microscopic observations the so called light line is found. The microhardness of this layer of seeds during their maturation increases from 45 to 80 kg mm^{-2} (Krakhmalev and Sultanova, 1983).

Comparison between microhardness of various peel layers (Krakhmalev and Gershman, 1990) and microhardness of known metals has shown, that for most mature cotton seeds, as, for example, for 108-F cotton sort, the external epidermis, the external pigmental and crystalliferous layers have approximately the same hardness, as annealed aluminum and the columnar layer at a level of a light line- as the annealed copper. Such feature of microhardness distribution among structural layers is also characteristic for the seeds of other cotton sorts (in sum, 30 types have been investigated) and it, apparently, has an essential influence on seed hardness or stoniness of the seeds. In roentgenographic investigations it has been found for selected mature seeds of the investigated cotton species with the laboratory germinating ability of 98%, that the shadow X-Ray images of the germs (roentgenograms) of such seeds (or germs) had uniform blackening on positive images. Only narrow parts of germ edges are slightly clarified as they have oval form. The germs occupy the seed cavities almost completely (Krakhmalev, 1991).

The following facts are very important for the technology of the seed treatment under hulling and pelleting. Every mature seed with the moisture of 8-10% and lower, always has narrow and hollow gap between seed coat and embryo (Fig.1a). Its size is 0.05-0.1 mm (Krakhmalev, 1991; Krakhmalev and Gershman, 1990). For immature seeds these gaps are significantly larger, though their external sizes can be close to external sizes of more mature seeds. According to Rakhimov and Rudenko (1976) dormant cotton seeds quantity varies from 2.0 to 4-8%. We have show that dormant seeds have morphological features of embryo (Fig. 1b). Their positive images in soft x-ray beams are characterized by various brighter strips. The decoded roentgenograms have shown that brightness of background is, apparently, related to internal structure of an embryo, to original arrangement of cotyledons and seed roots. The shadow images of the germs of hard seeds for different cotton sorts are characterized by their individual patterns of brightened shadow backgrounds of the germs (Fig. 2a-c). But in general, the most characteristic feature of the roentgenograms of hard, stony seeds is brightness around a rootlet of a embryo (Fig. 2d). Taking into account a nature X-Ray image formation of a embryo we can say that such locally brightened background on the roentgenograms is connected with smaller absorption of X-Ray beams in the given areas of embryo material, or is related to existence of free spaces-gaps between structural elements of a embryo. First assumption is related to the fact that the absorption of x-ray beams, transmitting through complex objects, is determined, in addition to their energy, by atomic composition, density and thickness of a sample (Krakhmalev and Sultanova, 1983). The heavier chemical elements entering into the object and the higher their density and thickness, the more intensive X-ray absorption takes place. As a result, a distinctive shadow image arises on photoemulsion after its appropriate processing (Burger, 1981). On the prints the images get opposite visibility; dark parts of a negative become bright on the photos. Thus, the roentgenogram of investigated objects differs in substance from its optical image obtained with optical microscope (Burger, 1981). The patterns are similar only by their contour borders. For the second assumption on occurred brightened parts on the photographic images it is necessary to recognize the presence of continuity breaks within the volume of germ body.

To explain the occurrence of contrast inhomogeneity on X-Ray images of stony cotton seeds, longitudinal and cross cuts of these seeds have been investigated. Among 65 stony seeds of five cotton kinds (Tashkent-1, 108-F, C-4727 *Gossypium hirsutum* L., C-6030, C-6524) 50 seeds were randomly selected for the embryo cut preparation. The distinctive features of the seed germ structures, which are characteristic for stony seeds have been found out. They are the gaps of various sizes inside a body of an embryo, which separate the rootlet off the seed-lobes. In this respect the cross cuts of the seed germs were the most informative (Fig. 3a-d). The gaps between a tissue of the cotyledons and the rootlets (cuts of the seed-roots in Fig. 3a-d were denoted by r) for some stony seeds were significant and well observable (Fig. 3a). For example, in Fig. 3a the size of large gap has the cross size of 1.5 mm by 1.0 mm and the size of smaller gap 1.0×0.3 mm. The gaps of very small sizes were also found with microscope (Fig. 3d, arrows). Their sizes were about 0.1×0.1 mm. Thus, the inhomogenity of X-Ray image structure on roentgenograms of cotton seeds is caused first of all by anomalous arrangement of the germ parts. Longitudinal cuts of stony seeds also show the presence of longitudinal slot-like hollows in a body of germs, which are often oriented along a conditional axis of a germ. The powerfully developed rootlet elastically supports the micropilar part of a peel, compressing the laid cotyledons in halasal part of a seed cavity. On longitudinal and cross cuts of stony seeds the spherical particles of gossypol are also seen. Using a technique of photography of the cut surfaces according to Krakhmalev *et al*. (1979), it is possible to determine the content of gossypol in separately taken cotton seeds.

The structural attribute of cotton seed hardness is clearly evident. But it is impossible to exclude the influence on this phenomenon of such fact, as occurrence of chemical heterogeneity in the structural elements of the germs and seed peel. The physiologists, dealing with cotton, know that the concentrations of the elements of inorganic nutrition are non-uniformly distributed: calcium

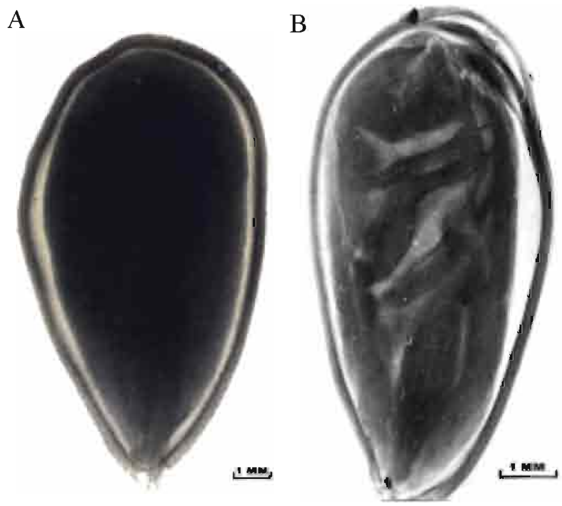

Fig. 1: X-Ray image of cotton seeds (positive image): a- Perfect seeds (uniform distribution of germ tissue density). Sort *Tashkent-1 G. hirsutum*; b- Stone seeds. We can see irregularity distribution of germ tissue density. Sort *Tashkent-1 G. hirsutum*

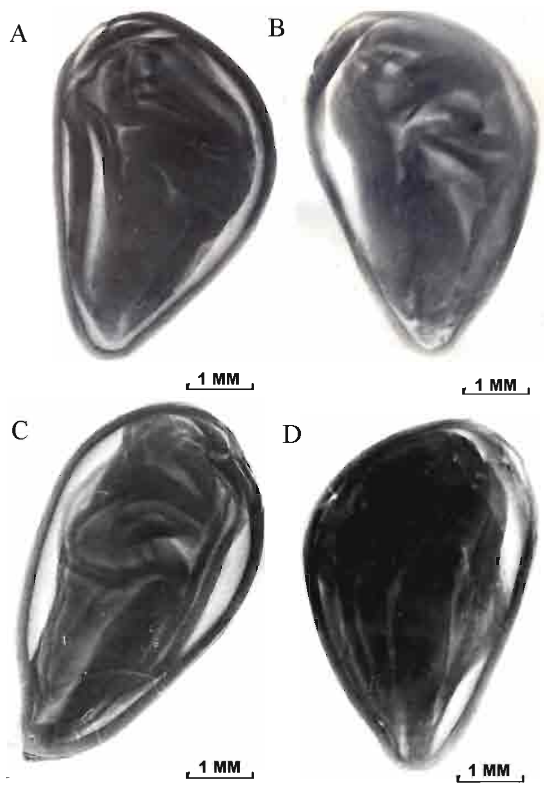

Fig. 2: X-Ray image of stone cotton seeds (positive image): a- Sort 108-F *G. hirsutum*; b- Sort C-4727 *G. hirsutum* c- Sort C-6030 *G.barbadense* L.; d- Sort Turfan guza *G. herbaceum* L.

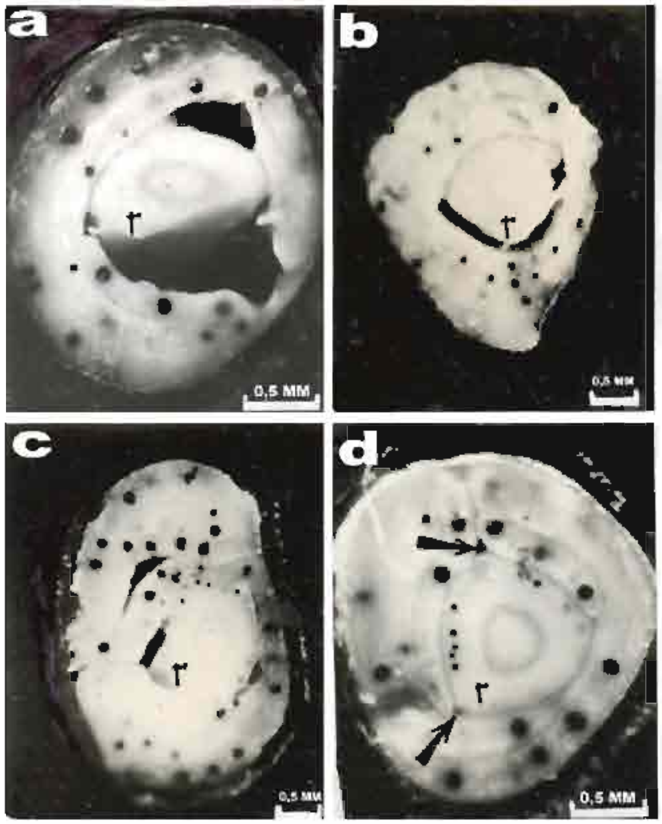

Fig. 3: Structure of the cross section surface of the stone cotton seeds germ (optical microscope): a- Sort Tashkent-1 *G. hirsutum*; b- Sort C-4727 *G. hirsutum*; c- Sort C-6030 *G. barbadense* L.; d- Sort Turfan guza *G. herbaceum* L. Cross sections of germ roots is designated as r. Arrows indicate to gaps small size between seed-lobes tissue and rootlet

and magnesium are accumulated in the leaf, in the folds of bolls, in seed peel; potassium prevails in a fibre; zinc, copper, phosphorus prevail in the seed nuclei. By the end of cotton vegetation more than 60% of total quantity of nitrogen, phosphorus and potassium, removed from the soil, pass into the seeds. Such non-uniformity in the element composition of cotton materials is easily found using the radioisotope methods of analysis in cotton industry (Grober, 1966). It is naturally to assume, that the increased concentration of various chemical elements in structural formations of the seeds results, for example, in abnormal jump of microhardness in the zone of a so-called light line (Krakhmalev and Sultanova, 1983). Probably, this fact and also the presence of inhibiting substances in cotton seeds (which are similar to the substances found in the seeds of *Astragalus tennesseans* (Baskin and Quarterman, 1969) and cause the obvious dormancy epicotyl's in a plant germ) give rise to the seeds become stony, though the germ is well developed and structurally differentiated.

The human eye has a resolution about 0.125 mm and we can see details of morphostructure of dormant cotton seeds on the roentgenograms. But now modern high-speed computers let us considerably shorten the time of measurement and increase its resolution (Krakhmalev *et al*., 1986). Therefore it is possible to develop such X-Ray device, which will allow to process 15-20 and more seeds per second. A technique of cotton seed radiography is very informative. In addition to determination of the seed hardness it is possible to use the roentgenograms in selection works for forecasting an opportunity of formation of such important seed defect in new cotton sorts, as peel with a fibre at primary processing of a raw cotton (Krakhmalev and Alimova, 1984). The radiography also is only one possible simple method allowing quality estimation of pelleted and sterilized seed grain of cotton (Krakhmalev and Gershman, 1986; Krakhmalev and Gershman, 1990).

CONCLUSIONS

The performed investigations of dormant cotton seeds *Gossypium hirsutum* L., *G. barbadence* L. and *G. herbaceum* L. by complex methods X-ray image, optical microscopy and seed coat microhardness method let us do the next conclusions:

- It is showed that dormant cotton seeds have aberrant morphological features of embryo such as gaps of various sizes inside of body of an embryo, which separate the rootlet from the seed-lobes. The gaps between the tissue of the cotyledons and the rootlets for dormant cotton seeds have sizes from 0.1 up to 1.0 mm.
- Hardness of cotton seed coat is determined by the line halasa - micropil and by tissues below this line. The greatest microhardness for mature cotton seed is observed in thin interlayer of seed coat, passing through top ends of columnar cells, where under the optical microscope the so called "light line" is found.

ACKNOWLEDGEMENTS

We would like to thank H. Abdullaev for excellent technical assistance during sampling. The research described in this paper was supported, in part, by a grant from the Center Science and Technology Republic of Uzbekistan (Contract P-17.64).

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