Early Development of Indonesian Cotton with Enhanced Resilience to Climate Change through Mutation Breeding

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Abstract. Indonesia is one of the top ten largest textile producer countries in the world. However, only 1-2 % of the national cotton demand was produced locally. The total value of imported cotton in Indonesia is nearly US$ 1 billion/year in 2017. Some of the production restraints were few cotton cultivars which are tailored specifically for Indonesian climate, only some regions of Indonesia are suited for cotton cultivation, and most cotton farmers rely on rainfed irrigation. Induced mutations are widely used for the introduction of genetic changes, particularly in crops that are not easily improved through conventional techniques. IAEA initiated a regional research project for cotton breeding in Asia region. Indonesia was invited to join this program in 2018, made it in total eight participating countries. Some genotypes from NIAB - Pakistan was distributed to all members as exchange germplasm to be utilized in adaptation trials and also used as genetic materials in each country breeding projects. At present, the Indonesian cotton mutation breeding is an ongoing program in M2 generation using irradiated three cotton cultivars (NIAB-KIRAN, NIAB-112 and NIAB-777).

1. Introduction
Cotton is one of the most important and widely produced agricultural and industrial crops in the world. It is grown in more than 100 countries, making it one of the most significant crops in terms of land use after food grains and soybeans. Cotton is also a heavily traded agricultural commodity, with over 150 countries involved in exporting or importing cotton [1]. Fang and Percy stated that although mostly known as fiber source, cotton has other functions [2]. The cotton lint, an industrial raw material and renewable agricultural resource, competes with synthetic fibers in the textile industry. Cotton seeds are pressed to produce edible oil, and serves as the world’s second most important oilseed. The seed meal and hull provides additional protein nutrition to cattle and poultry feed. Furthermore, the utilization of cotton lint is not limited only for textile. Kavilanz mentioned that since natural fibers, such as cotton and linen, can bind stronger than cellulose, the paper made with both fibers can bear the mistreatments plain paper cannot [3]. Each US dollar bill for example, is made of 75 % cotton and 25 % linen. Indonesian rupiah also incorporates cotton fiber within its paper bill [4].

Lee and Fang explains that there are four domesticated species of cotton. \(G\)ossypium \(a\)rboreum \(L\). and \(G\). \(h\)erbaceum \(L\)., both diploids, are native to the Old World [5]. \(G\). \(a\)rboreum remains an important crop in India, while \(G\). \(h\)erbaceum, is today grown mostly for local use in the drier areas of Africa and Asia. \(G\). \(b\)arbadense \(L\). and \(G\). \(h\)irsutum \(L\)., both allotetraploids, evolved in the New World. \(G\). \(b\)arbadense, commonly known as the Egyptian cotton, supplies 3 - 5% of the current fiber world production. Its fiber is mostly used for the luxury fabrics and sewing thread production. Meanwhile \(G\). \(h\)irsutum, or more widely known as Upland cotton, contributes about 95% of the world production.
Cotton can be grown in both tropical & sub-tropical conditions (Figure 1). The cotton plant is a woody perennial shrub that is grown as an annual field crop [5]. According to Chaudry, it required a minimum temperature of 15°C for proper field germination [6]. The optimum temperature for vegetative growth is 21-27°C. High temperature up to 43°C is tolerable, but temperature below 21°C is detrimental to the crop. Warm days and cool nights, with big diurnal variations during the fruiting period are essential to good boll and fiber development. Depending on the climate & crop-growing period, cotton generally needs 700-1,200 mm water to meet its water requirement.

![Fig. 1. The relationship between the latitude and climate of 73 cotton producing countries and the average fiber yield (kg/ha) in each country in 2014. ICAC World Cotton Database (https://icac.generation10.net), adapted from Conaty et al. [7].](image)

Indonesia is one of the top ten world’s largest textile and garment producer, but only around 1-2 % of the national cotton demand was fulfilled by local production. About 42 % of Indonesian textile production are using cotton as raw material. The national demand for cotton reaches 550,000 million tons/year. The total value of imported cotton in Indonesia is nearly US$1 billion/year [8]. The thriving Indonesian textile industry, starting from the thread spinning business, whether using traditional or modern machinery, until the garment and ready-to-wear clothes industry, absorbs a significant number of workers and actively supporting the economy. Therefore, in order to conserve of national foreign exchange reserve, it is essential to increase the local cotton production.

Cotton growing regions in Indonesia are limited. For its cultivation, cotton plants need adequate amount of water from germination until vegetative stage. During flowering and fruit filling cotton can tolerate drought to some degree. But after the fruits matured and the bolls starting to crack, exposing the fibers inside to the environment effects. The ready-to-harvest cotton plantations are vulnerable to the slightest rainfall as it can greatly damage and ruin the fiber quality. However, Indonesia is an archipelago spread across the equator, and situated between two oceans which provides high precipitation all year round (see Fig. 2). This leads to warm and humid climate for most of the country, and prone to the erratic tropical rains. Hence, most of cotton producing regions in Indonesia are mostly located in the drier, eastern part of the country, such as East Java, West Nusa Tenggara, East Nusa Tenggara, and South Sulawesi, which climate are more influenced by Australia.
For cotton improvement, Chaudry [6] mentioned that basically there are three methods: introduction, selection and hybridization. Varieties were imported from one country and directly adopted in another. This is probably the shortest and easiest way to improve the genetic background. The cotton production itself was initiated by introduction in the Indian subcontinent, although there might be some adaptation issues. Selection within a population has also proved successful. The major limitation has been insufficient variability, as the existing variability was resulted from out-crossing or natural mutations. Because of the limitations, efforts were made to induce variability through mutagenesis [10]. Percy et al. [11] said that mutant traits were at first regarded as interesting oddities, but their potential were recognized as heritable traits. Among the first mutants to be described was the colored lint trait, followed by the okra leaf trait [12], and the red leaf [13]. However, radiation was extensively used in many countries. For cotton, gamma rays were more effective than other radiation sources for desirable mutations [6].

Up to now, there are 15 national cotton varieties released by BALITTAS (Indonesian Sweetener and Fiber Crops Research Institute), located in Malang, East Java. Kanesia 8 is the current national standard variety: production potential 1.85 - 2.73 ton seeded cotton/ha and fiber percentage 33.3 - 38.7%. The latest cotton cultivar release was in November 2018, namely Bronesia 1 & 2, named after the natural brown color of the cotton fiber [14]. In the past, CIRA-NNEA has released one mutant cotton variety, named Kharisma-1 in 2007, featuring high fiber density and high yield (2 tons/ha) without additional insecticide spray. However, the cotton mutation breeding program in the institution was closed in 2009.

The idea of IAEA RAS 5075 program was formulated in Rio De Janeiro, Brazil during the International Cotton Conference in 2016. Several scientists working on cotton mutation breeding in Asia developing countries initiated the idea of working together within a cooperation research program, under IAEA, to develop new varieties of cotton that are more resilient to the climate change effects. Pakistan, who is the most advanced in term of cotton mutation breeding agreed to be the country leader. The members in 2016 were Bangladesh, Myanmar, Syria, Iran, Vietnam and China. After one year into the program, Vietnam and China dropped off the program. Indonesia was invited to join the aforementioned project in early 2018, together with two more Southeast Asia countries: Thailand and Cambodia.

The object of this study is to initiate a new cotton mutation breeding program in each participating country (in this case in Indonesia) using introduced cotton germplasm from Pakistan under IAEA RAS 5075 program.
2. Materials and Methods

2.1. Development of M1 population and adaptation test

Indonesia has received seeds of in total six cotton germplasm, namely NIAB-112, NIAB-KIRAN, NIAB-452, NIA 2008, NIAB 777, and NIAB-825 in April 2018. Before planting in the field, the samples of these introduction seeds were subjected to a preliminary germination test in the greenhouse facility of CIRA-NNEA in Pasarjumat, South Jakarta, Indonesia. Twenty seeds of each genotype were directly germinated on the moist soil on a metal tray, and kept in the greenhouse for two weeks. Unfortunately, it was found that the germination rate of those seeds were very low, perhaps due to primary or secondary seed dormancy.

The second germination trials were tested in June 2018, using pre-germination treatment of soaking the seeds overnight with sterile water, in order to break the seed dormancy. The pre-treatment managed to improve the germination rate, although not high as expected. Therefore, it was decided to irradiate only three best germination cultivars (NIAB-KIRAN, NIAB-112, and NIAB-777) and grown the rest of the introduced germplasm in the field as an adaptation test. Before planting, all seeds were soaked overnight to induce better germination in the field.

Three cotton cultivars that were received from NIAB- Pakistan (NIAB-KIRAN, NIAB-112 and NIAB-777) has been irradiated with gamma ray, using dosages of 200 and 300 Gy, respectively. The first generation (M1 plants) were grown in June 2018. Regular daily irrigation was carried out especially during the first two months after sowing. The experiment plot was also cleaned out regularly for weed every month. Pest and disease control were executed according to the condition in the field. The harvesting the cotton bolls were done in many batches since cotton plants did not ripened all fruits simultaneously. The bolls started to crack open since September 2018 and continued up to April 2019.

2.2. Gamma irradiation orientation dose test

The orientation dose is usually done before any breeding program was started, in order to find out the optimum dose of the plant material. Even for the same crop or species, different cultivar or genotype could have different radio sensitivity response against gamma ray irradiation. The cotton seeds of NIAB KIRAN were packed in plastic clip, each containing 60 seeds. Irradiation was carried out in CIRA-NNEA facility of Gamma Cell 220, each with doses of 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 Gy. Afterward, seeds were sown in metal tray, 20 seeds per row, and using 3 replications. Observation of germination rate, plant height, number of leaves, and chlorophyll index were done 2 weeks after sowing. The chlorophyll index is measured with SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan), which calculates the SPAD value based on the intensity of light transmitted around 650 nm (red band) where absorption by chlorophyll is high and a reference wavelength around 940 nm (Markwell et al, 1995). SPAD was measured from three points (upper, middle and lower parts of a leaf) and were averaged to represent individual measurement of a leaf. The sample leaf used for measurements was the youngest fully expanded leaf.

2.3. M2 in-vitro screening for drought tolerance

In mutation breeding, selection can already begin since the M2 population. This experiment was conducted as screening for tolerance level of the cotton seeds from M2 generation to the drought stress, utilizing Polyethylene Glycol (PEG) solution in tissue culture medium. The PEG concentration could influence the survival rate of the germinated cotton seeds, since PEG are widely use to imitate the drought stress condition in the in-vitro culture of other agricultural crops [15].

In order to speed the screening process for tolerance against drought character, another experiment was started in February 2019. This ongoing research is part of a bachelor student thesis using the plant material of harvested M2 seeds of three cotton cultivars: NIAB-KIRAN, NIAB-112, and NIAB-777 of 200 and 300 Gy, and also seeds from non-irradiated plants as control. The seeds were germinated on petri dishes size 100 x 200 mm containing MS medium. Upon germination, the seeds were transferred to PEG containing medium (0, 10 and 15% of PEG respectfully) for one month of observation. The M2 seeds harvested from one M1 plants are treated as replicates. Each petri dish contain three explants.
The seeds used as the plant materials were separated manually by hands from the cotton fibers. Cotton seeds harvested from the field were fuzzy in appearance, since they are still covered in lints, even after separated from the fibers. The attached lints trapped dirt and fungi spores from the field, made the sterilization process of the cotton seeds an enormous task. Several sterilization protocols for cotton seeds were tried, and a modified sterilization protocol based on Afolabi-Balagun et al was finally utilized [16].

2.4 Development of M2 population

The seeds that were harvested from M1 plants were called M2 seeds, and when grown into a plant, the plant will be called M2 plant [17]. M2 seeds have been harvested from M1 plants irradiated with 200 Gy dose, while most of the 300 Gy M1 plants, were late for entering the flowering stage and produced very few seeds. Fruits both from control and M1 plants were collected in separate bags per individual plant. The harvested seeds are dried for some weeks in our seed processing facility. Since our old cotton ginning machine (purchased in 2006) for separating seeds from cotton fruit shells and fibers are often malfunctioning, most of the works are conducted manually, separating by hands. This process takes a lot of time and labor, and therefore the data are not shown in this paper.

Some parts of the M2 seeds were used in the M2 in-vitro screening using PEG solution (see section 2.3). From the in-vitro culture that were contaminated in the process, the M2 explants were acclimatized in the greenhouse, and then transferred to grow in the pots.

The M2 seeds that were completing the processing stage, properly labelled, was then sown on the Pasarjumat Experiment Field, South Jakarta, Indonesia on 15 August 2019. For each plot sized 3 m x 1.5 m with planting distance 25 x 50 cm for each holes. The material used were M2 seeds from NIAB KIRAN, NIAB 112 and NIAB 777 of 0 and 200 Gy. Unfortunately, M2 seeds of 300 Gy are very low in number and mostly abnormal. Fertilizer used were NPK Mutiara of 16-16-16 applied 2 weeks after sowing.

3. Results and Discussion

3.1. Development of M1 population and adaptation test of introduced germplasm

Nuclear Institute for Agriculture and Biology (NIAB), based in Faisalabad, Pakistan dispersed two of their own varieties (NIAB-KIRAN and NIAB-414) to each participating countries to be subjected to the observation and adaptation trials, grown together with two local cotton cultivars in each country as comparison. Since each participating countries has different climate and sowing season, the advance of the trials also varied among them. The farthest progress was achieved in Myanmar, who already reached M7 generation. Situated in near equator with favourable climate, the cotton researchers in Myanmar are able to plant 2-3 times per year. This step has proved very successful as they advanced onto M5 generation. In some other sub-tropical countries, planting season can only be executed 1-2 times per year. For example, the cotton mutation breeding program in Syria just reaches M3 generation.

Cotton in general is not suited to the tropical climate hence the cultivation in Indonesia are limited [7]. In the more fertile regions of Indonesia, farmers choose to grow vegetables or other plantation crops such as rubber or coffee. Cotton requires high and long sunlight intensity to reach optimal production capacity. The unpredictable rains in the most parts of the country could heavily damage the cotton harvest. These obstacles led to low national cotton production.

The center of production is mostly limited to the eastern regions of Indonesian archipelago, which in general relies heavily on rainfed irrigation. The cotton cultivation in these areas are vulnerable to the effects of adverse climate change, and there is a need for cotton varieties that are adapted to these stress conditions. Conaty et al. said that the challenge to growing cotton in typical long humid region is the variability of cloud cover and rainfall during early and mid-fruiting [7]. In this tropical environment, cotton is usually sown during the wet season, then matures and is harvested in the dry season. The practice ensures that the vegetative stage of crop is completed during the rainfall period, and bolls mature during periods of high quality radiation.

As part of RAS5075 project, Indonesia has received six cotton mutant genotypes as germplasm exchange from NIAB – Pakistan in March 2018 (NIAB-KIRAN, NIAB-777, NIAB-112, NIAB-846,
NIAB-2008, and NIAB-852). The seeds of six genotypes were sown in the CIRA-BATAN experiment field trial in South Jakarta, Indonesia on 26 July 2018. Preliminary trial has been conducted in April 2018 to check the seed viability of the six earlier genotypes. It turned out that the seed viability of these introduced cotton genotypes was very low, ranged from 10-30%.

In order to overcome the low germination rate problem, the seeds were soaked in sterile water overnight prior to sowing in the field. The strategy has improved the seed germination rate, but nevertheless two times more sowing repetitions, executed in interval of two weeks after the previous sowing, in order to have a considerable field trial population size. In each sowing, 100-150 seeds were planted in the soil. Each plot was consisted of 5 rows, 20 x 50 cm distance. Two seeds were planted for each hole. The agronomic performance was given at Table 1 below. It can be concluded that NIAB KIRAN are performed best in adaptation test in Pasarjumat experiment field trial, and followed by NIAB 777 and NIAB 112.

### Table 1. Adaptation test for six cotton mutant cultivars from NIAB-Pakistan

| No | Genotype    | Germination rate (%) | Plant height (cm)       | Number of branches |
|----|-------------|----------------------|-------------------------|--------------------|
| 1  | NIAB KIRAN  | 36.3                 | 159.40 ± 25.66          | 27.43 ± 8.64       |
| 2  | NIAB 852    | 33.7                 | 109.62 ± 20.64          | 4.84 ± 3.39        |
| 3  | NIAB 846    | 18.3                 | 126.74 ± 16.09          | 8.82 ± 6.16        |
| 4  | NIAB 777    | 20.3                 | 133.73 ± 19.40          | 20.20 ± 8.58       |
| 5  | NIAB 2008   | 18.1                 | 131.70 ± 28.28          | 10.08 ± 7.19       |
| 6  | NIAB 112    | 15.7                 | 140.00 ± 22.38          | 16.15 ± 3.93       |

Regardless of the low germination rates, the surviving plants from six cultivars could all grow well and bear fruits in Indonesian climate. The best growth is shown by NIAB KIRAN, NIAB 112 and NIAB 777 which has best plant height and number of branches (Table 1). Although NIAB 852 could germinated relatively well compared to others (33.7%), the plant stature was short and having very few effective branches (4.84) to bear fruits. Many plants of this cultivar produced very few or even no fruits at all until the end of experiment.

Since the harvesting time is coincident with the rainy season, it happened that for few months the production was completely stopped (November 2018-February 2019). Cloudy conditions combined with high night temperatures may reduce yield potential [18, 19]. Conaty et al. [7] assumed that it may be caused by increased number of aborted fruits, together with less production of fruiting sites resulted from reduction of carbon assimilates supply and increased respiration rates. The abscission of flowers, and young bolls, as also happened in our field, is a survival mechanism. Conaty et al. [7] further explained that in the tropics, this response is associated with low radiation period, where the plant reduces its internal demand for assimilates by shedding developing fruit. The plants revert to the vegetative stage, then later produce bolls when conditions are more favourable. In our case, flower and fruit production was resumed in late February 2019 as the rainfall lessened.
Table 2. Agronomical performance of three introduction cultivars after irradiation

| Genotype   | 200 Gy | 300 Gy |
|------------|--------|--------|
|            | No. of plants | Plant height (cm) | No. of branches | No. of plants | Plant height (cm) | No. of branches |
| NIAB-KIRAN | 66     | 131.22 ± 35.94 | 14.22 ± 12.57 | 25           | 115.27 ± 37.48  | 15.42 ± 13.40  |
| NIAB-777   | 85     | 140.65 ± 33.95 | 20.68 ± 12.28 | 52           | 90.38 ± 12.99   | 6.41 ± 3.34    |
| NIAB-112   | 61     | 99.74 ± 30.14  | 5.16 ± 3.93   | 28           | 85.04 ± 10.72   | 4.80 ± 2.93    |

From the two gamma ray irradiation dose (200 and 300 Gy), the difference in term of plant growth is quite significant. The plants grown from 200 Gy dose seeds have better performance in all three cultivars (NIAB KIRAN, NIAB 777 and NIAB 112), although in term of number of branches, NIAB KIRAN plants with 300 Gy irradiation has more branches (15.42) compared to the 200 Gy (14.22 branches). It seems that NIAB KIRAN has higher level of tolerance against gamma irradiation compared to the other two cultivars, although NIAB 777 seeds could germinated better than both NIAB KIRAN and NIAB 112 in 300 Gy population.

Although the number of seeds sown are the same (around 300 seeds), but the number of surviving plants are varied. Not all plants of 300 Gy irradiation can produced seeds, since many bolls produced were having abnormal, wrinkled seeds. Compared to 200 Gy population, the number of normal, viable seeds obtained from 300 Gy population are limited. The flowering time was also delayed by 2 months later from the 200 Gy population.

![Figure 3] Some morphological changes found in M1 population post irradiation treatments

There are some morphological changes that were observed in M1 population, due to the effect of irradiation treatment. Some examples are shown in Fig. 3 below. It was observed that one individual plant of irradiated NIAB-KIRAN dose 200 Gy were growing larger and taller than the surrounding plants (Fig. 3A). Unfortunately, the same plant also exhibited partial sterility with more than half of its fertilized fruits failed to develop further and get aborted (Fig 3B). Partial albino leaves (Fig. 3C) are common phenomenon be found in M1 population after irradiation. The existence of albino leaves, whether whole or partial, is often used as visual indication for plant mutation breeders that irradiated induced mutation has taking place in the treated M1 population [17]. Beside changes in leaf color, the leaf shape is also often malformed as in Fig 3D, especially on the first few leaves that grow on the first month after sowing. The leaves that were showing up in later stage are usually reverted back to have normal leaf shapes.
3.2 Gamma irradiation orientation dose test

Another study of cotton irradiation dose orientation was conducted in January 2019, using NIAB-KIRAN seeds as plant material. The seeds were subjected to ten levels of gamma irradiation (0, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 Gy subsequently) and sown on soil tray. Each irradiation dose was represented by 20 seeds as samples, and three replicates. The plant growth responses were measured at 2 weeks after sowing. The results were shown in Fig. 4 and Table 3 below.

![Observation on cotton orientation dose for gamma irradiation treatments 2 weeks after sowing](image)

**Table 3.** The agronomic data of cotton genotype NIAB-2008 on two weeks after gamma irradiation treatment

| Irradiation dose (Gy) | Average plant height (cm) | Average no. of leaves | Average root length (cm) | Average chlorophyll index |
|-----------------------|---------------------------|-----------------------|--------------------------|--------------------------|
| 0                     | 13.13                     | 3.00                  | 19.38                    | 45.50                    |
| 100                   | 10.64                     | 3.00                  | 17.50                    | 42.30                    |
| 200                   | 10.50                     | 3.00                  | 19.00                    | 36.70                    |
| 300                   | 10.30                     | 2.67                  | 21.00                    | 43.83                    |
| 400                   | 6.42                      | 2.00                  | 11.70                    | 45.72                    |
| 500                   | 6.40                      | 2.00                  | 6.33                     | 51.27                    |
| 600                   | 6.90                      | 2.00                  | 13.20                    | 34.46                    |
| 700                   | 4.07                      | 1.67                  | 8.67                     | 46.10                    |
| 800                   | 3.75                      | 2.00                  | 6.50                     | 46.70                    |
| 900                   | 2.70                      | 2.00                  | 4.00                     | 55.70                    |
| 1000                  | 2.45                      | 2.00                  | 5.00                     | 61.50                    |

The higher the irradiation dose received by the seeds, usually the higher damage they will show during growth. It can be seen from Table 3 that the best growth is shown by 0 Gy plants, however up to 300 Gy, the average plant height at two weeks after sowing are still above 10 cm. The root length was even the best at 300 Gy (21 cm), although not much differ from the lower doses. But at 400 Gy
and above, the root length was drastically reduced. It can be assumed that the optimum dose for cotton seeds are between 200 to 300 Gy.

Shresta et al., explained that the chlorophyll index indicated the density of chlorophyll content within the leaf tissue [20]. Plants suffered from irradiation treatments were often having darker color leaves and tend to have more concentrated chlorophyll content on the leaves along with the higher irradiation dose (see Table 3). These changes were often temporary, and this character most probably will not be passed to the next generation.

3.3 M2 in-vitro screening for drought tolerance
Seeds that are harvested from M1 population are called M2 seeds.

Figure 5 Optimization of sterilization protocol for cotton seed in-vitro culture initiation

Several sterilization protocols for cotton seeds [16, 21, 22] were tested in the biotechnology laboratory from February-April 2019 to obtain the suitable protocol for contamination removal. Despite trying out those established protocols, the contamination level of the explants was still high (Figure 4).

The modified cotton seed sterilization protocol was finally established as below. In order to remove the remaining lint from the seeds, at first the seeds were soaked in sulfuric acid H2SO4 solution for about a minute, stirred gently, then taken out from the solution by long lancets. This work should be done strictly in a fume hood, and the workers must wear protective gloves and special mask in order to protect themselves from the strong acid. After being washed three times with sterilized water, the seeds were dried out overnight on opened Petri dish in room temperature. On the next day, the seeds were being washed one more time, then soaked in sterilized water in a closed container for three days. On the third day of soaking, the seeds were peeled out from the seed coat. In the laminar air flow, the peeled seeds were soaked with 10% chlorox, washed several times, and then transferred on MS medium. The result is so far superior with nearly 100 % no contamination. After the seeds germinated, they would be sub cultured to PEG containing medium. We will be expecting the result of this PEG in-vitro screening within three months’ period.

3.4. Development of M2 population and future plans
There are some laboratories scale equipment given as a grant from IAEA (Liliput Cotton Ginning Machine, and Handheld Cotton Picking Machine) that up to now are still in the shipment process. Their arrival would be a great help in order to hasten the harvest and seed processing.
Some contaminated M2 seeds of NIAB KIRAN, NIAB-777 and NIAB-112 from M2 in-vitro screening experiments were acclimatized to grow ex-vivo in the greenhouse, about 20-25 plants of each cultivar (Fig. 6). These M2 plants are various stages, some already ready for harvest, and some are now entering the flowering stage. Large variation is observed in term of plant growth and performance. Beside these potted plants, a new field experiment was set up recently (sown on 15 August 2019) for M2 population of NIAB KIRAN, NIAB 112 and NIAB 777 of both 200 Gy and control plants.

Figure 6. M2 cotton population in pots and newly sown in the field

According to Chaudry [6] cotton breeding is in a transition stage in which the way that genetic principles are applied is changing. Continual support of expertise and technical assistance is needed through participating in accepting expert mission and training courses, especially to improve the breeding methodology. The project has established a great network of regional cotton improvement researchers, and Indonesia would benefit a lot from it.

Since the country is situated on the equator, Indonesia has erratic and unpredictable tropical rain climate, which can delay the flowering and fruiting of the cotton plants. Greater Jakarta or West Java provinces are not exactly suitable location for cotton cultivation. Therefore, the optimal growth and production of the cotton germplasm introduction from NIAB-Pakistan cannot be attained. The cotton field trials were in the peak of harvesting season when the rainy season was starting, and the constant heavy rain has temporarily halted the subsequent flowering and fruiting process, made it difficult to have an accurate estimation of yield or production.

The Indonesian government has assigned another research institution under Ministry of Agriculture, BALITTA, which is located in Malang, East Java province, to conduct research in fibre and sweetener crops, including cotton. The climate in Malang is much more suitable for cotton cultivation. Contacts have been made to include them as research counterpart in this RAS 5075 project, and the subsequent field trials will be conducted in this area.

4. Conclusion
The IAEA RAS 5075 is a four years project (2016-2019), and has four main targets, i.e. human capacity building, initiation of mutation breeding program in participating countries, evaluation of existing germplasm in participating countries, and networking. Some of the achievements in Indonesia (2018-2019) are the completion of adaptation test of six introduced Pakistan germplasm to local climate, with three cultivars (NIAB KIRAN, NIAB 112 and NIAB 777) are proved to be well adapted to Indonesian climate. A good size of M1 mutant population from these 3 cultivars also has been developed as the material for further cotton breeding program. Screening in vitro of M2 population for drought tolerance are ongoing, and M2 population are underway for screening in the field. The scope of this program beside the cotton germplasm exchange is also as a forum to exchange information and experience about cotton mutation breeding techniques, including the selection techniques in order to obtain superior mutant lines up until ready to be release as new cotton varieties and being used by farmers in each countries. Although institutions facilitating germplasm exchange for research purpose was well established for several other crops in the world (eg. rice, wheat etc.), there is none available
yet for cotton. The regional Asia cotton breeder network which was built through this program would be a valuable source of knowledge for any future cotton research in Asia.

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