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In-vitro Hybridization of Tomato (*Solanum lycopersicum* L.)

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Abstract

Tomato (Solanum lycopersicum L.) hybrid variety trials were conducted at "Directorate of vegetable seed production farm Quetta" where two varieties of tomato (Riogrande & Dolor) were evaluated on the basis of days to flowering, fruit setting, length and width of fruit, average fruit weight, plant height , yield, maturity period and producing new hybrid variety which is suitable for climate prevailing in Quetta. The development strategy was "Inter-varietal Hybridization" and hybrid progeny often have a higher yield, increased resistance to disease, and an enhanced performance in different environment compared with the parental lines. Cost effective mechanism to create commercial hybrid seed parental line is one of the vital components which ultimately examine the commercial viability of hybrid varieties. Emasculation was aimed at first generation (F1) hybrid seed utilization globally in term of salient cultivars and volume of utilized seed. (F1) hybrid seed can be characterized into manual pollinated and gene control pollinated species. After careful research study of tomato hybrid seed production in Quetta was illustrated different production steps from selection of site to actual growing of the male and female parents, emasculation of the male flowers pollination, eventually we got a suitable tomato hybrid for the Quetta and other similar climatic regimes.

Keywords: Inter-varietal Hybridization, yield potential, maturity period, F1 hybrids, hybrid Seed Production Review.

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INTRODUCTION

Tomato technically termed as (*Solanum lycopersicum* L.) is the edible, often red fruit berry of the Solanaceae or nightshade family. It is believed that tomato species are originated from South America to other parts of the world. Many varieties of tomato are now widely grown in greenhouses and other cooler climates of the globe. Tomato is utilized in variety of methods including raw, as an ingredient in many dishes, sauces, salads and drinks. Botanically tomato is a fruit rather than a vegetable which is extensively used for culinary purposes. A typical tomato fruit of 100g (3.5 oz) size possess energy (18 kcal), carbohydrates 3.9 g, sugar 2.6 g, fat 0.2 g, protein 0.9 g, water 94.5 g, vitamin B6 0.08 mg , vitamin C 14 mg, vitamin E 0.54 mg and vitamin K 7.9 ug .

Wild species of tomato originated in the Andean area of South America, probably in Peru and Ecuador regions and is thought to be domesticated in various parts of Mexico long before the arrival of Europeans.

Generally tomatoes are profusely branched, spreading about 60-180 cm recumbent when fruiting, but a few farms are compact the upright. Botanically describing features of leaves which are more or less hairy, strongly odorous, innately compound and grown up to 45 cm long. Flowers are yellow, 2-3 cm across, pendant and clustered. Tomato fruits vary in diameter from 1.7 to 7.5 cm and are usually red, prickly vary in shape almost spherical through over and elongate to pre-shape. Tomato fruits are soft, succulent berry, containing two to many cells of small seeds surrounded by jelly like pulp which is enriched with vitamin C. Usually tomato is

served raw in salads, cooked as vegetable, as an ingredient of various prepared dishes and pickled in various flavors (Britannica).

Tomato is a popular fruit vegetable in many parts of the world. It is particularly rich in pigments secondary metabolites like lycopene (red pigment in tomato fruit) - a strong anti-carcinogen and Vitamin C. (Mishra, 1998). It is widely grown although consumption and preparation methods differ in various countries. In Zambia tomatoes are cooked as a relish, on its own or stewed with onions or are used as a condiment of almost all relishes (Sanjoy *et al.,* 1999). In Zambia, much of the tomato is produced for the fresh market and consumed as a component of relishes an important vegetable grown by both commercial and small scale farmers in open fields. The physical and chemical characteristics of the soil or artificial media, the greenhouse internal environment and the nature of the plant (cultivar selection) determine the most critical productivity parameters for plant growth (Sanjoy *et al.,* 1999).

Tomato is an herbaceous annual with a creeping stem covered with single hairs. The plants are characterized either as indeterminate or determinate types based on plant habit and vigor. The determinate types ultimately develop a bunch of flower cluster at the terminal point of growth causing plants to stop at certain growing height (Sanjoy *et al.,* 1999).

Determinate tomatoes are bushy and usually stop growing at about 1.5 m (Messmer, 2010). The leaves are compound and alternate. The flowers are borne in inflorescences of 4 – 6 yellow flowers. The fruits are in a variety of shapes; round, elongated, cylindrical and oval or pear shaped and in varying sizes (Rida e*t al.,* 2002).

In Zambia some of the common varieties grown include Rodade, Heinz, Money maker, Star 9030 and Tengeru (Moya e*t al.,* 2000). Rodade is semi-determinate, bearing very firm, medium sized fruit, have long bearing period and are resistant to Bacterial wilt as well as Verticillium and Fusarium (Sharma and Rastogi, 1993). Heinz is medium-sized, medium firm fruit, borne on plants providing good cover and resistant to cracking (Sharma and Rastogi, 1993). Star 9030 is an indeterminate tomato hybrid with vigorous growth for winter production. It has good resistance against disease for summer production and has above average fruit size (Sharma and Rastogi, 1993) engeru has a long growth period up to over 12 months and take 3 months from planting to harvest. They grow up to 2.5 meters tall and yields between 25 and 50 tons/ha (Sanjoy *et al.,* 1999).

Under high intensity precision horticultural production systems, artificial media is used instead of direct soil planting because plants grow well in media that will hold water as evenly as possible and provide sufficient nutrient holding capacities. Un even uptake of water can cause many problems including damping off, flower drop, fruit splitting and blossom end rot (Rida, e*t al.,* 2002). Greenhouses provide environmental control that includes both the soil and above ground micro environment. Agriculture is an important and growing sector of the Zambian economy (Muthuvel et al., 2000.).

Parent lines and climate of Balochistan

An open-pollinated heirloom determinate variety originates from Italian region and have been cultivated in Balochistan since 14 years. As we know that Riogrande is a Non-GMO variety of tomato, it's a naturally growing variety. Riogrande is cultivating throughout the world and the effectiveness of Riogrande varies among different climates of the world so according to the climate of Balochistan it has many advantages and disadvantages.

Advantages

Riogrande is high fruiting tomato variety has medium fruit size. Attractive plum shape and red color of fruit. Shelf life of fruit is 10 days at room temperature. Average maturity time 75-85 days.

Disadvantages

Riogrande is a very dwarf plant among determinate varieties. Plant height is 2.7-3.5 feet's. During irrigation many of its fruit destroy. Riogrande gives high production at those areas who's temperature range is between 43 to 47 degree Celsius. Suitable soil for Riogrande is loamy soil. Dolor is a self-

pollinated hybrid determinate Variety originates from Mexico. And have been cultivating in Pakistan since 9 years. Dolor is a very effective variety of tomato especially for high altitude areas but this variety also has some advantages and disadvantages.

Advantages

Show maximum growth and give high production at areas like Balochistan. Fruit colour is pink red and have attractive pear shape tomato. Have maximum maturity time and can available in market for long time.

Disadvantages

Dolor is a taller plant among determinate variety. For better production we have to provide support and intensive care is necessary. Shows better growth and production in Balochistan, but it is an average fruiting plant. Shelf life of its fruit is very short 5 days at room temperature.

Climate of Balochistan

A fairly dry and arid climate prevails in Quetta and most parts of the Balochistan. District Quetta is situated at an altitude of 1,700 meter above sea level. Resultantly, weather is severely cold and dry. Winters are usually very cold and the minimum temperature ranges between the regimes of 15 to -7 °C. Whereas summers are relatively mild and the maximum temperature ranges from 32 to 35 ºC. Soil type of Quetta is sandy loam and alkaline in nature having pH more than 7 and calcareous in nature.

MATERIALS AND METHODS

We used two types of materials.

1. Living material
2. Non-living material

Living material

Tomato plants with flowers to serve as the female and male parents. Riogrande (male parent) Dolor (female parent)

Non-living material

Seed starting mixture
Breeding kit
Fine scissor
Tweezers
Electric Brush
Black Plastic Sheet
Tags and Bags
Note Book
Shadow covering
Protective sticks and plastic wires
Plastic sheets
10 % ethanol solution
Vernier caliper
Fertilizers
Pest control
Weighing scale

Methods

We used these methods to complete the research work.

- Experimental site
- Experimental design
  Nursery
- Organic soil fertility management

Results Riogrande Dolor Nursery sowing 11-02-14 11-02-2014 Germination 20-02-2014 21-02-2014 Transplanting to normal sunlight 05-03-2014 05-03-2014 Transplanting to open field 25-03-2014 25-03-2014

1st Flowering	03-05-2014	13-05-2014
1st Fruiting	29-05-2014	09-06-2014
1st Picking	25-06-2014	09-07-2014
2nd Picking	15-08-2014	07-09-2014

0 Bed preparation
0 Transplanting
0 Irrigation
0 Mounding
0 Staking
0 Pruning and tying
0 Mulching
0 Pest and disease control
0 Selection of parents
0 Emasculation & Bagging
0 Pollen collection
0 Crossing
0 Self-pollination

- Tagging
- Harvesting
- Seed extraction and Processing

RESULTS & DISCUSSION

From the beginning of experiment till experiment end you have to be careful and keep each and every thing in a order as it's performed.

We have grown two different determinate varieties in which one is hybrid Dolor (Female) and other is heirloom Riogrande (Male).

Inter-varietal hybridization is performed to get a F1 hybrid generation for measuring our results parameters. And later on this F1 hybrid is intercrossed and we evaluate F2 after that remaining parameters are compared with parent lines.

Table 1: Germination of tomatos

Results	Riogrande	Dolor
Nursery sowing	11-02-2-14	11-02-2014
Germination	20-02-2014	21-02-2014
Transplanting to normal sunlight	05-03-2014	05-03-2014
Transplanting to open field	25-03-2014	25-03-2014
1st Flowering	03-05-2014	13-05-2014
1st Fruiting	29-05-2014	09-06-2014
1st Picking	25-06-2014	09-07-2014
2nd Picking	15-08-2014	07-09-2014

This table shows the Germination time of both varieties and also their flowering, fruiting, and picking day's differences.

The table number 2 shows the germination rate of Riogrande is 88 % and of Dolor is 91 %.

Also tells us the Number of flowers tag during research and how much successful fruits we get from our experiments.

Table 2: Experimental calculation

Results	Riogrande	Dolor
No.of Seed sowing	70	70
No.of Germinates	62	64
Transplanted	55	58
Selection of parents	35	45
Self-pollinated flowers in 1st picking	30	30
Successful Self-pollinated fruits in 1st picking	27	28
Self-pollinated flowers in 2nd picking	30	30
Successful Self-pollinated fruits in 2nd picking	26	27
Crossed flowers in 1st picking	8 (pollen collection)	30
Successful Crossed fruits in 1st picking	Nill	22
Crossed flowers in 2nd picking	8 (pollen collection)	30
Successful Crossed fruits in 2nd picking	Nill	25

Table 3. Comparison of F1 hybrid with F1 parents

Results	F1 Riogrande	F1 Dolor	F1 hybrid
Fruit size	12*17 (avg L*W) Medium (3.5 oz)	10*14 (avg L*W) Medium (2.9 oz)	12*16 (avg L*W) Medium (3.4 oz)
Fruit color	Red	Pink (red)	Red
Fruit shape	Plum	Pear	Mixed shape (not plum not pear)
Shelf life	11 days at room temperature	6 days at room temperature	10 days at room temperature

This table is comparison of F1 hybrid with F1 parent lines.

Size of the fruit is also improved regarding to their parents.

In F1 generation hybrid gain the color of parent father

F1 hybrid got mixed shape that we can say exactly plum or pear

F1Dolor

Riogrande

Shelf life of hybrid fruit is also improved regarding to their parents
We adjust the temperature of room between 22 to 25 ºC.
At this stage of research we achieved the objectives that includes Fruit size,color, shape and most importantly shelf life of hybrid fruit.
And for achieving other objectives like:
a) Plant height b) Maturity time c) fruiting type d) Producing new variety:-
As we know that these both parent lines are determinates in nature and they give production annually so, I extract and store the seeds of F1 hybrid.
Subsequently, we had grown F1 hybrid and also check the nutrient requirements of F1 hybrid and its ability to survive in the climate of Quetta, that either our F1 hybrid is able to survive and give maximum production in Quetta climate or not.
Then F1 and F2 were inter-crossed lately they were evaluated. From the same population of F1 hybrid we selected few plant that serve as male and other healthy plants that will serve female plants and then intercrossed them and evaluate F2.
After the evaluation of F2 we were able to compare our remaining parameters with their F1 parent lines (Riogrande & Dolor) like hybrid plant height, its fruiting style fruiting type and maturity time of hybrid plants.
On the other side we had grown parent lines for comparison purposes.
Subsequently, we had grown F2 evaluate F3 and the same procedures repeat again and again till F7 and also till lines.
After getting lines we performed Genetic analysis and check the genetic makeup of hybrid line and then compare it with parent lines to ensure that, either the characteristics of our in trust are transferred in hybrid lines from parents or not. After that we got a new hybrid variety.

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