Growing new markets with better bitter gourd genetics:

WorldVeg's monoecious, gynoecious and predominantly female lines of different market segments developed through recurrent selection to breed breakthrough hybrids

Research proposal

For

APSA-WorldVeg Vegetable Breeding Consortium

By

World Vegetable Center

World Vegetable Center

Proposal Summary

Project title	**Growing new markets with better bitter gourd genetics:** WorldVeg's monoecious, gynoecious and predominantly female lines of different market segments developed through recurrent selection to breed breakthrough hybrids	
Submitted to	APSA-WorldVeg Vegetable Breeding Consortium Members	
Main WorldVeg contact person	Mandy Lin (mandy.lin@worldveg.org)	
Main WorldVeg scientists and designation	Dr. Narinder Dhillon, Principal Plant Breeder – Cucurbits	
Project duration	2 years (1 January 2023 - 31 December 2024)	
Project fees (US$)*	730,000	Minimum of 25 companies at 29,200 USD per company
		Maximum of 35 companies at 20,857 USD per company

**The participating seed companies will equally share Project fees.*

Objectives

The overall objective is to enable members of the APSA-WorldVeg Vegetable Breeding Consortium to access the elite bitter gourd lines developed through recurrent selection and F1 hybrids for developing market competitive F1 hybrids resulting in sustainable breeding gains and enhanced profitability of bitter gourd production for smallholder farmers. The specific objectives are:

1. To test the performance of WorldVeg's advanced bitter gourd breeding lines bred by the recurrent selection, including gynoecious and predominantly female lines.
2. To test the performance of WorldVeg F1 hybrids of bitter gourd developed by using WorldVeg's elite lines and new sources of resistance to *Tomato leaf curl New Delhi virus* (ToLCNDV).
3. Organize annual Bitter Gourd Open Field Days to showcase the specific horticultural traits of WorldVeg's unique breeding lines and F1 hybrids and make available the seed of entries selected by the project member seed companies.
4. Develop single nucleotide polymorphism (SNP) markers associated with gynoecy quantitative trait loci (QTLs) and with genetic loci conferring resistance to powdery mildew and ToLCNDV.

Background

Bitter gourd (*Momordica charantia* L.) is one of the important commercial cucurbitaceous cash crops grown mainly by smallholder farmers in Asia where it is cultivated on more than 340,000 ha annually (McCreight et al., 2013). The current bitter gourd seed market in Asia values ≈Euros 16 million (Narendra Kumar Singh, formerly with HM Clause, personal communication). In India alone, the bitter gourd seed annual market is 530-580 MT (F_1 hybrid seed = 250-280 tons and OP seed = 280-300 tons;

Dhillon et al., 2020). Bitter gourd fruit abounds in phytonutrients (Dhillon et al., 2017) and is often used in folk medicine to manage type 2 diabetes. Today, 537 million people (20-79 years) live with diabetes. Type 2 diabetes account for nearly 90% of all diabetes cases (IFD, 2021) and 80% of those are in low-income and middle-income countries. This figure is projected to rise to 643 million by 2030 and 783 million by 2045. Further, 541 million adults are at increased risk of developing type 2 diabetes. Clinical studies confirmed that diet supplementation with bitter gourd fruits lower elevated fasting glucose in pre-diabetics (Amirthaveni et al., 2018; Krawinkel et al., 2018).

Bitter gourd cultivars 'Jade Star' and 'Vivek' were the first F1 hybrids released in the early 1980s in the Philippines and India by East-West Seed and Mahyco, respectively, and this breakthrough transformed bitter gourd in Asia from a semi-commercial crop into a major commercial crop. More seed companies initiated bitter gourd breeding programs, often using a small number of closely related bitter gourd lines in commercial breeding, focusing mainly on elite x elite crosses to capitalize on previous breeding success which resulted in a narrowing of the genetic diversity among the bitter gourd commercial cultivars, a situation confirmed by Simple Sequence Repeat (SSR) analysis at World Vegetable Center (Dhillon et al., 2016). The low genetic diversity of elite bitter gourd cultivars rendered the crop vulnerable to biotic and abiotic stresses, and limited prospects for long-term yield improvements.

WorldVeg has bred more than 400 bitter gourd breeding lines of different commercial segments through inbreeding of selected WorldVeg genebank accessions (heterogeneous and heterozygous) originating from different Asian countries, and subsequent selection within and among progeny rows. These lines exhibited improved yield and enhanced fruit quality in WorldVeg trials and at other locations. Some of these lines have been rated as "field resistant" to cucurbit powdery mildew (*Podosphaera xanthii*), downy mildew (*Pseudoperonospora cubensis*) and *Tomato leaf curl New Delhi virus* (Dhillon et al., 2018; Laenoi et al., 2019; Yadav et al., 2019). This elite bitter gourd germplasm was made available to the member seed companies of WorldVeg bitter gourd projects in phases 1-3 of the project. This partnership has been very successful in widening the pool of genetic resources used in the seed companies' commercial breeding programs and introducing new traits such as begomovirus resistance, powdery mildew resistance, and high yield (Dhillon et al., 2020).

A novel bitter gourd recurrent selection program was initiated at WorldVeg in 2011. The base population was created by intercrossing diverse genebank accessions and commercial hybrids. The population possesses important horticultural traits such as high fruit yield, fruit glossiness, uniform green fruit without white tips and narrow necks, early bearing, long fruit duration, and resistance to cucurbit powdery mildew and begomovirus. The pedigree selection followed four cycles of recurrent selection. Bitter gourd lines developed from this unique program are now being tested in observational trials. These new lines have many key traits such as blunt strong fruit spines/smooth in some segments (traits desired by distributors and consumers), uniform shining green fruit color, absence of white tips and narrow neck (traits preferred by consumers), improved early and total yield, and resistance to cucurbit powdery mildew and begomovirus (traits desired by the growers).

Use of gynoecious or predominantly female lines in bitter gourd heterosis breeding results in early, high yielding F1 hybrids, especially in small and medium fruit market segments, as demonstrated by WorldVeg and a few private seed companies (Dhillon et al., 2020). WorldVeg has developed 6 gynoecious and 8 predominantly female bitter gourd lines classified into small and medium fruit market segments of South Asia types.

Methods/Activities

Activity 1. Conduct trials of WorldVeg bitter gourd lines developed through recurrent selection and produce breeder seed of these entries

Evaluate 200 elite bitter gourd lines (100 lines per year) at the WorldVeg East and Southeast Asia Research and Training Station, Kasetsart University, Kamphaeng Saen, Thailand. Ten plants of each line will be transplanted into single 9.6 m^2 plots. The following horticultural traits will be recorded and the lines' fruit picture database will be created:

1) days to 50% pistillate flowering after transplanting;
2) days to 50% staminate flowering after transplanting;
3) fruit length;
4) fruit breadth;
5) fruit weight;
6) fruit number/vine;
7) fruit weight;
8) fruit skin pattern–warts/smooth;
9) fruit appearance– glossy/non-glossy;
10) field tolerance to cucurbit powdery mildew;
11) field tolerance to begomovirus;
12) fruit bitterness–bitter/non-bitter.

Activity 2. Test the performance of WorldVeg gynoecious and predominantly female lines developed through recurrent selection and breeder seed production

Evaluate 5 gynoecious and 5 predominantly female lines of bitter gourd. Ten plants of each line will be transplanted into single 9.6 m^2 plots. Horticultural traits as mentioned in Activity 1 will be recorded.

Activity 3. Evaluate new powdery mildew resistant lines derived from landraces through pedigree selection

Fifteen new powdery mildew resistant lines will be evaluated for horticultural traits as mentioned in activity 1 and for their reaction against a local powdery mildew strain (*P. xanthii*) through spreader rows in the field.

Activity 4. Evaluate new bitter gourd lines resistant to *Tomato leaf curl New Delhi virus* (ToLCNDV) for horticultural traits and against ToLCNDV at a virus hotspot in India

Twenty new sources of resistance to ToLCNDV will be evaluated for horticultural traits as mentioned in Activity 1 and data on their reaction to ToLCNDV at a virus hotspot in India will be generated.

Activity 5. Evaluate new Gaz Karela (long, firm fruit) lines derived through pedigree selection from Bangladeshi landraces

Evaluate thirteen Gaz Karela bitter gourd lines for horticultural traits as mentioned in Activity 1. These lines have the potential to develop long segment and firm fruit products.

Activity 6. Evaluate new promising lines derived through pedigree selection from Asian landraces

Evaluate 163 new lines for horticultural traits as mentioned in Activity 1.

Activity 7. New base population for a new round of recurrent selection

Produce seed of a base population for different market segments using genetically diverse lines belonging to two different heterotic groups including lines with excellent fruit color and firmness and lines resistant to leaf curl virus and powdery mildew.

Activity 8. Evaluate WorldVeg's experimental F1 hybrids of bitter gourd

Evaluate 40-50 WorldVeg's experimental F1 hybrids of bitter gourd at various stages of testing (observational yield trial/OYT, preliminary yield trial/PYT, advanced yield trial/AYT) each year at the WorldVeg East and Southeast Asia Research and Training Station, Kasetsart University, Kamphaeng Saen, Thailand. Ten plants of each entry will be transplanted into single 9.6 m^2 plots and there will be two and three replications for PYT and AYT, respectively. Horticultural traits as mentioned in Activity 1 will be recorded.

Activity 9. Mapping of gynoecy QTLs and of genetic loci conferring resistance to powdery mildew and ToLCNDV in bitter gourd

Detailed Information on SNP markers associated with these QTLs and resistance loci will be provided. The Genotyping-by-sequencing platform will be used to generate the SNP markers.

Activity 10. Organize Bitter Gourd Open Field day

WorldVeg Bitter Gourd Open Field Day will be held each year in July at the WorldVeg East and Southeast Asia Research and Training Station, Kasetsart University, Kamphaeng Saen, Thailand. Seed companies contributing to this project will be invited to observe the performance of bitter gourd lines and F1 hybrids and select the candidate lines and hybrids for their breeding program. Each participating company will be provided 30 elite breeding lines free of charge every year.

Deliverables

#	Description
1	New elite lines of various market segments derived through recurrent selection (200)
2	New gynoecious lines derived through recurrent selection (5)
3	New predominantly female lines derived through recurrent selection (5)
4	New powdery mildew resistant lines derived from landraces through pedigree selection (15)
5	New sources of resistance to Tomato leaf curl New Delhi virus (20)
6	New Gaz Karela (long, firm fruit) lines derived through pedigree selection from Bangladeshi landraces (13)
7	New promising lines derived through pedigree selection from Asian landraces • South Asia type, long segment (21) • South Asia type, medium segment (68) • South Asia type, short segment (6) • Vietnam type (29) • Southeast Asia type (29) • White fruit segment (10)
8	Seed of a base population (for different market segments) developed using leaf curl and powdery mildew resistant lines, excellent fruit color and firmness lines; the lines used are genetically diverse belonging to two different heterotic groups so that seed companies can start their own

	recurrent selection program in their defined environment to derive elite inbred lines of various segments for developing future hybrids
9	SNP markers associated with loci conferring resistance to powdery mildew
10	SNP markers associated with loci conferring resistance to Tomato leaf curl New Delhi virus
11	SNP markers associated with gynoecy QTLs
12	40-50 WorldVeg's experimental F1 hybrids in Observational Yield Trial, Preliminary Yield Trial and Advanced Yield Trial each year for participants observation and selection

Duration and budget

The project will be conducted from 1 January 2023 to 31 December 2024 with the following budget:

Budget item	Cost (US$)
Personnel	443,620
Supplies & operations	153,977
Travel	20,000
Sub-total	617,597
Indirect costs (18.2%)	112,403
Total	730,000

Timeline of activities

Activity	**2023**	**2024**
Field trials conducted for activity 1	√	√
Field trials conducted for activity 2-6	√	
Seed of new base population for new round of recurrent selection made available; activity 7		√
Field trials conducted for activity 8	√	√
Bitter Gourd Open Field Day organized at WorldVeg Thailand	√	√
Mapping of QTLs conferring resistance to powdery mildew, ToLCNDV, and gynoecy trait		√
Seed shipped to the seed companies contributing to the project	√	√

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