In vitro Screening of Potato (*Solanum tuberosum* L.) Genotypes for Osmotic Stress Tolerance

Dandena Gelmesa[1], Nigussie Dechassa[1], Wassu Mohammed[1], Philippe Monneveux[2], Christin Hadden[3] and Traud Winkelmenn[3]

[1]School of Plant Sciences, Haramaya University, Ethiopia; [2]International Potato Center, Lima Peru, [3]Institute of Horticultural Production Systems, Woody Plant and Propagation Physiology, Leibniz University Hannover, Germany

WORLD CONGRESS ON ROOT AND TUBER CROPS

World Congress on Root and Tuber Crops
Nanning, Guangxi, China, January 18-22, 2016

- **Potato (*Solanum tuberosum* L.) in the world**
  - ❖ Number one non-grain food community (Rykaczewska, 2013)
  - ❖ Third most important food crop in the world after rice and wheat (Hancock, *et al.*, 2014; Birch *et al.*, 2012)
  - ❖ Important food security (Birch *et al.*, 2012) crop in developing countries
  - ❖ Play significant role to the human diet
    - Starch, antioxidants, protein,
    - vitamins, macro and micronutrients,
  - ❖ Source of income for small holder farmers and private sectors

- Potato is becoming an important crop in African because of its "hunger-busting" potential.
- **Able to feed Africa in the era of climate change (http://phys.org/news/2013-05-potato-ethiopia-era-limate.html)**

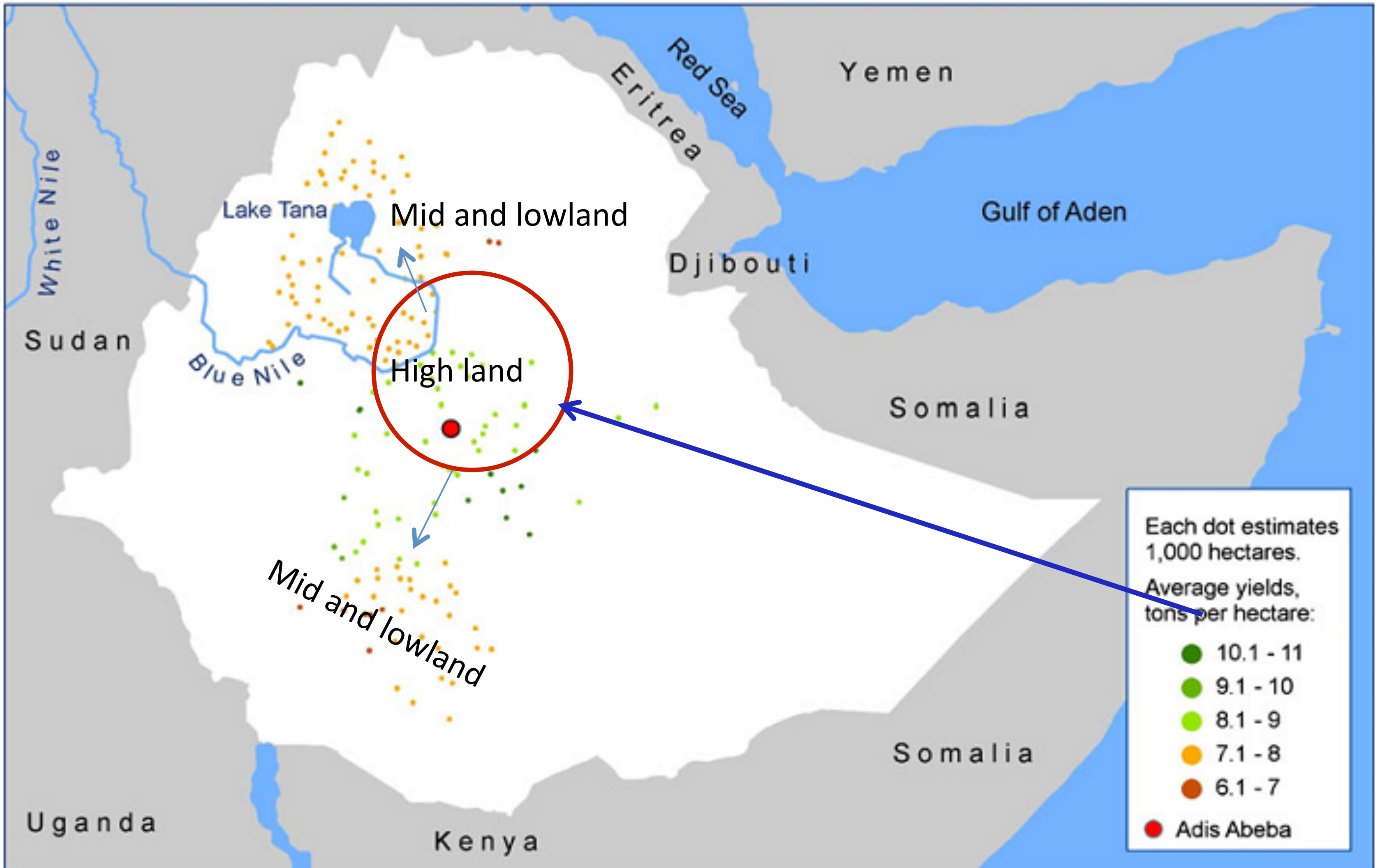
- Grown by more than 1.5 million farmers in Ethiopia (CSA, 2014)

- In the last 20-30 years, area planted with potato increased from 30,000 ha to 164,146 ha (CIP, 2011; Muthoni *et al*., 2011) in Ethiopia

- However, productivity remains very low due to certain factors

- Some improved varieties have been released for **high yield, wide adaptation, resistance to diseases** (Gebremedhin *et al.* 2008, Baye Berihun and Gebremedhin, 2013)

Potato production areas vs national yield average in Ethiopia



Source: http://research.cip.cgiar.org/confluence/display/wpa/Ethiopia. Hirpa *et al*., 2010

4

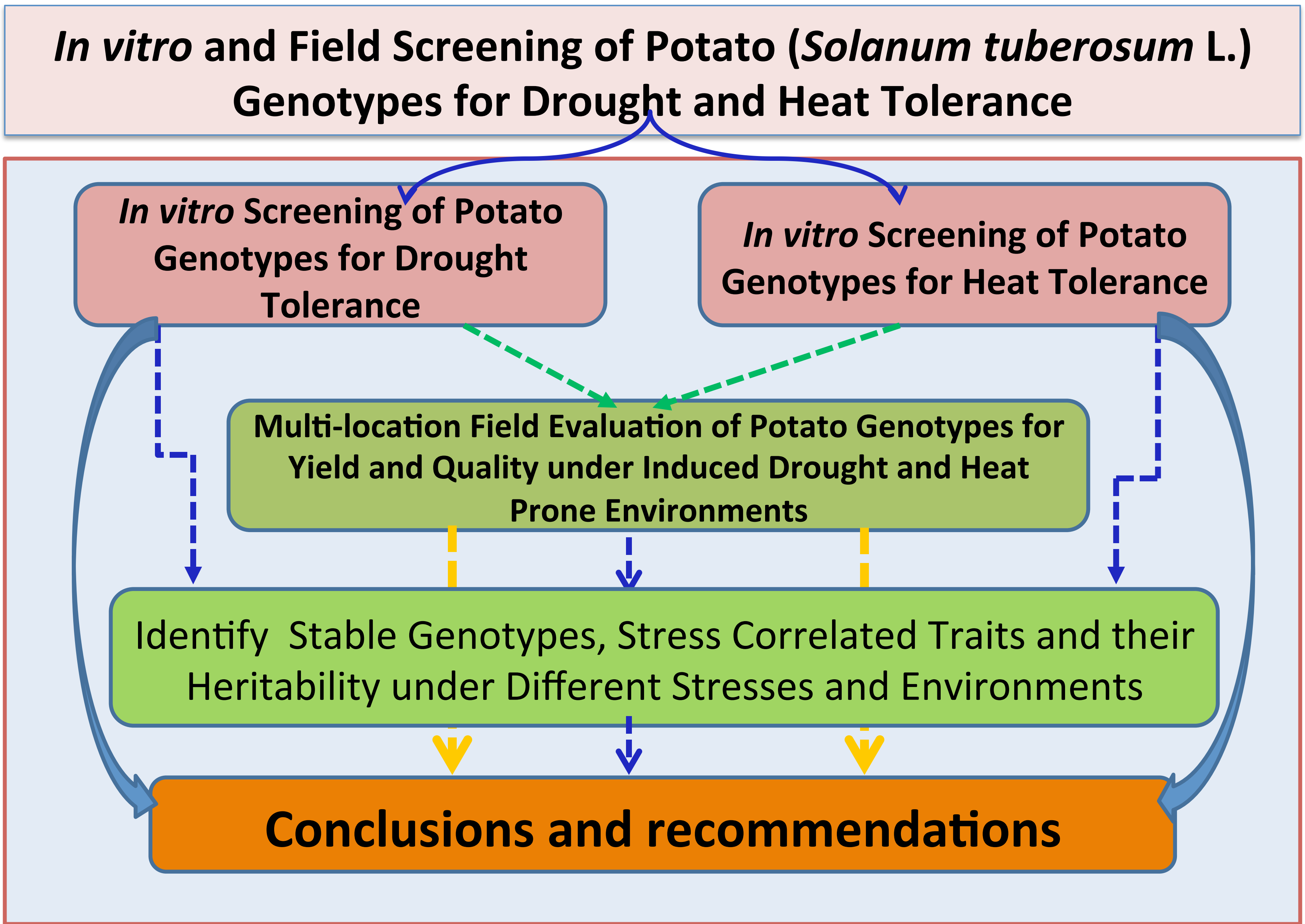
Potato production shifting to off season

- From rain fed based production to irrigation based production
  - Off-season production is around 78% of total potato production (Haverkort, 2012)
- Production has been expanding to **MIDLAND** and **LOWLAND** areas
  - ❖ *Used for production after other staple crops are harvested*
  - ❖ *Income generating potential to small scale farmers*
  - ❖ *Increased consumption of potato products*
  - ❖ *Short season crop and high return per unit area*
- Abiotic stresses such as drought and high temperature are the main production challenges farmers have experienced
  - ❖ Contributing to significant yield reduction world wide (CIP, 2009)

Central Rift Valley-Ethiopia (1620 masl)

Eastern Ethiopia-performance of different varieties

Adaptation trial at lowland areas, 2012


In vitro and Field Screening of Potato (Solanum tuberosum L.) Genotypes for Drought and Heat Tolerance
In vitro Screening of Potato Genotypes for Drought Tolerance
In vitro Screening of Potato Genotypes for Heat Tolerance
Multi-location Field Evaluation of Potato Genotypes for Yield and Quality under Induced Drought and Heat Prone Environments
Identify Stable Genotypes, Stress Correlated Traits and their Heritability under Different Stresses and Environments
Conclusions and recommendations

In vitro screening experiment

- To identify drought tolerant genotypes , morph-biochemical traits responsible for drought tolerance, heritability and association among the traits

1. CIP collection of lowland potato genotypes
   - Virus resistant nursery
   - Virus resistant and drought tolerant nursery
   - Virus resistant and heat tolerant nursery
2. Improved potato varieties grown in Ethiopia
3. Local potato varieties
   - Largely grown in mid and lowland areas
   - Collected from individual farmers or
   - Seed producers cooperates

Table 1. List of drought and or heat tolerant potato clones introduced from CIP and used for *in vitro* screening to osmotic stress tolerance.

Entry No	Accession ID	Origin	Pedigree	Traits reported
2	CIP 302499.30	CIPHQ	720139 x 392820.1	VR,HT
3	CIP 303381.106	CIPHQ	388611.22 x 676008	VR,HT
4	CIP 303381.30	CIPHQ	388611.22 x 676008	VR,DT
6	CIP 304350.100	CIPHQ	CHIEFTAIN x 392820.1	VR,DT,HT
7	CIP 304350.18	CIPHQ	CHIEFTAIN x 392820.1	VR,DT
8	CIP 304366.46	CIPHQ	392823.4 x 676008	VR,DT
9	CIP 304368.46	CIPHQ	391846.5 x 676008	VR,HT
11	CIP 304371.20	CIPHQ	MONALISA x 92.187	VR,DT,HT
12	CIP 304371.67	CIPHQ	MONALISA x 92.187	VR,DT,HT
13	CIP 304383.80	CIPHQ	800824 x 92.187	VR,HT
14	CIP 304387.39	CIPHQ	REINHORT x 92.187	VR,HT
15	CIP 304394.56	CIPHQ	SHEPOOY x 391207.2	VR,HT
16	CIP 304405.42	CIPHQ	WA.018 x 676008	VR
17	CIP 304405.47	CIPHQ	WA.018 x 676008	VR,HT
18	CIP 304406.31	CIPHQ	WA.077 x 676008	VR,DT,HT
19	CIP 388615.22	CIPHQ	B-71-240.2 x 386614.16	VR,HT
20	CIP 388676.1	CIPHQ	378015.18 x PVY-BK	VR,HT
21	CIP 388972.22	CIPHQ	386316.1 x 377964.5	VR,HT
22	CIP 390478.9	CIPHQ	720087 x 386287.1	VR,HT
23	CIP 392745.7	CIPHQ	88078 x 386316.1	VR,HT
28	CIP 395436.8	CIPHQ	388615.22 x 388615.22	VR,DT
29	CIP 396311.1	CIPHQ	391925.2 x C92.030	VR
30	CIP 397006.18	CIPHQ	389468.3 x 88.052	VR,DT
31	CIP 397016.7	CIPHQ	92.119 x 88.108	VR
32	CIP 397036.7	CIPHQ	392011.1 x 392745.7	VR,DT
33	CIP 397077.16	CIPHQ	392025.7 x 392820.1	VR,DT
34	CIP 397079.6	CIPHQ	386768.10 x 392820.1	VR,DT

CIP-International Potato Center; HQ-Head Quarter; VR-Virus Resistant; DT-Drought Tolerant; HT-Heat Tolerant

Table 2. Improved potato cultivars selected for in vitro screening

Variety	Suitable altitude (masl)	Yield (t ha^{-1})		Breeding center/seed source
		On station	On farm	
Bubu	1650-24330	39.5		Haramaya University
Belete	1600-2800	47.2	28-33.8	Holeta Agricultural Research Center
Gudene	1600-2800	29	21	Holeta Cgricultural Research Center
Bulle	1700-2700	39.3	38.3	Awassa Agricultural Research Center
Challa	1700-2000	42	35	Haramaya University
Gorebella	2200-3200	30-52	26-30	Sheno Agricultural Research Center
Jalane	1600-2800	40.3	29.10	Holeta Agricultural Research Center
Zemen	1700-2000	37.2	-	Haramaya University
Chiro	1600-2000	32-40	25-35	Haramaya University

Table 3. Local potato cultivars selected for in vitro screening

Variety name (local)	Maturity days	Tuber yield (t/ha)	Area of Collection (ZONE)	Seed source
Bate	86	28.5	Haramaya district	Rare Hora Seed Producer Cooperative
Dadafa	80-90	38.7	Haramaya district	Rare Hora Seed Producer Cooperative
Jarso	80	30.2	Haramaya district	Rare Hora Seed Producer Cooperative
Local Chiro	85	47.2	Kersa district	Haqan Gudina Seed Producer Coop
Tulema	90	36.5	Kersa district	Haqan Gudina Seed Producer Coop
Samune	80	28.9	Chiro district	Abdi Jalela Seed Prodcuer Coop
Methehera	-	-	Gemechis district	Bilisa Seed Producer Coop

Methodologies

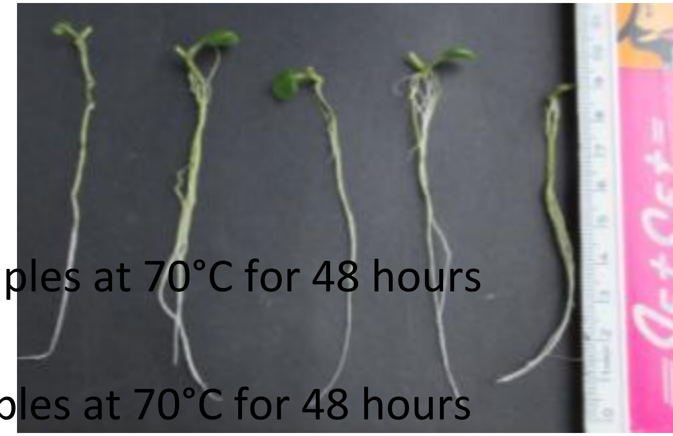
- Murashige and Skoog (1962) medium was used
- Water potential of the media reduced by addition of D-Sorbitol
  - Control = corresponding to -0.8 MPa Ψ_w
  - 0.1 Molar D-Sorbitol (18.219 g/lit) ≈ -1.1 MPa Ψ_w
  - 0.2 Molar D-Sorbitol (36.438 g/lit) ≈ -1.35 MPa Ψ_w
- A stem cutting of 1-1.5 cm length having one leaf and one node from the middle portion of the plantlets were used
- 6/5 plantlets were cultured per vessel for each treatment and replicated eight times in two round repetition
- Factorial arrangement of 1032 treatment combinations in RCD were used and incubated *in vitro* in TC lab for 30 days before data collection
- 16 hrs photoperiod of 3000-4000 lux light intensity at 18±2 °C constant temperature was maintained

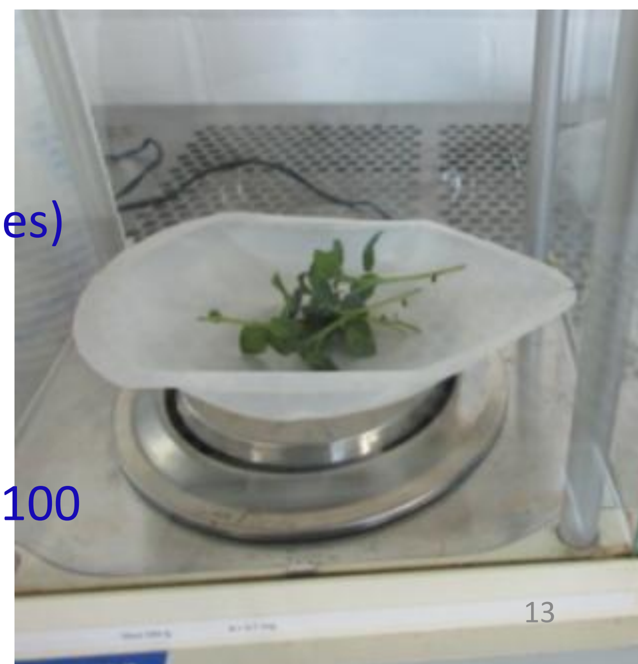
Measurement of drought related traits

1. Days to root initiation
2. Days to shoot initiation
3. Leaf number per plantlet
4. Shoot length (cm)
5. Shoot fresh weight (mg plantlet^{-1})
6. Shoot dry weight (mg plantlet^{-1})-drying fresh samples at 70°C for 48 hours
7. Root fresh weight (mg plantlet^{-1})
8. Root dry weight (mg plantlet^{-1})- drying fresh samples at 70°C for 48 hours
9. Root number plantlet^{-1}
10. Root length (cm)
11. Proline content (for selected genotypes)

– And Calculation of

- Root-to-shoot ratio (Fresh and Dry weight bases)
- Fresh Biomass (mg plantlet^{-1})
- Dry Biomass (mg plantlet^{-1})
- % Moisture content
- Relative response (%)-RR=(drought)/control)*100
- Stress susceptibility Index (SSI)





Data analysis and Interpretation

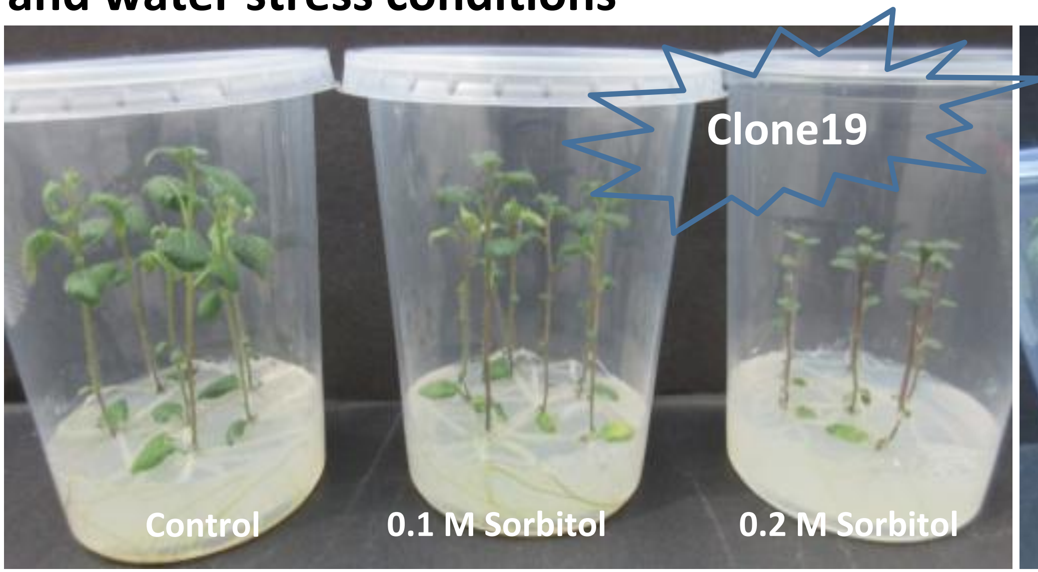
- Analysis of variance (ANOVA)
- Multivariate correlation & regression analysis
  - $r = \frac{Covariance\ XY}{\sqrt{varianceX\ x\ varianceY}}$
  - Liner regression (Y=a+bX)
- AMMI/ GEI interaction
- Stress susceptibility Index ($SSI=\frac{[1-(Y0.2M\ sorbitol/Ycontrol)]}{[1-(\bar{Y}0.2M\ sorbitols/\bar{Y}control)]}$)
- Cluster analysis ($EDjk = \sqrt{\sum_{i=1}^{n}(Xij - Xik)^2}$)

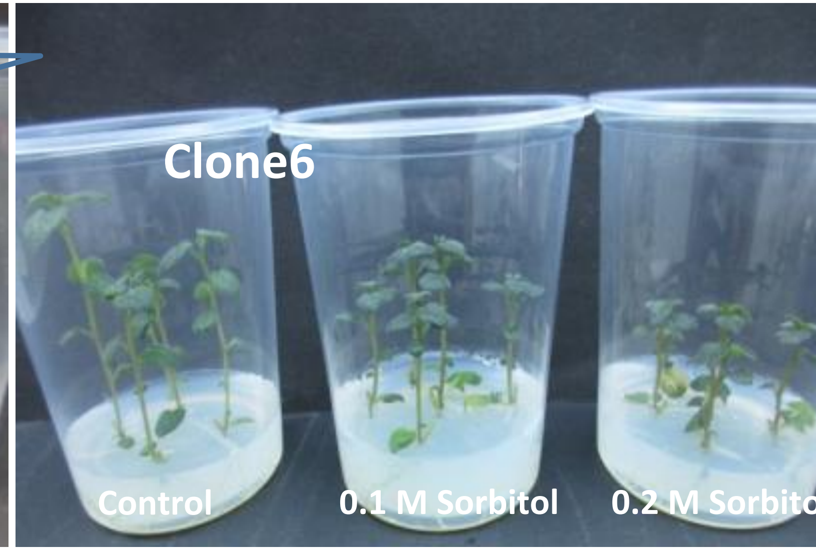
$$RR(\%) = \frac{\text{Shoot dry weight yiled of genotypes at stress-environment (0.2M Sorbitol)}}{\text{Shoot dry weight yield of genotypes at non-stress environment}} x100$$

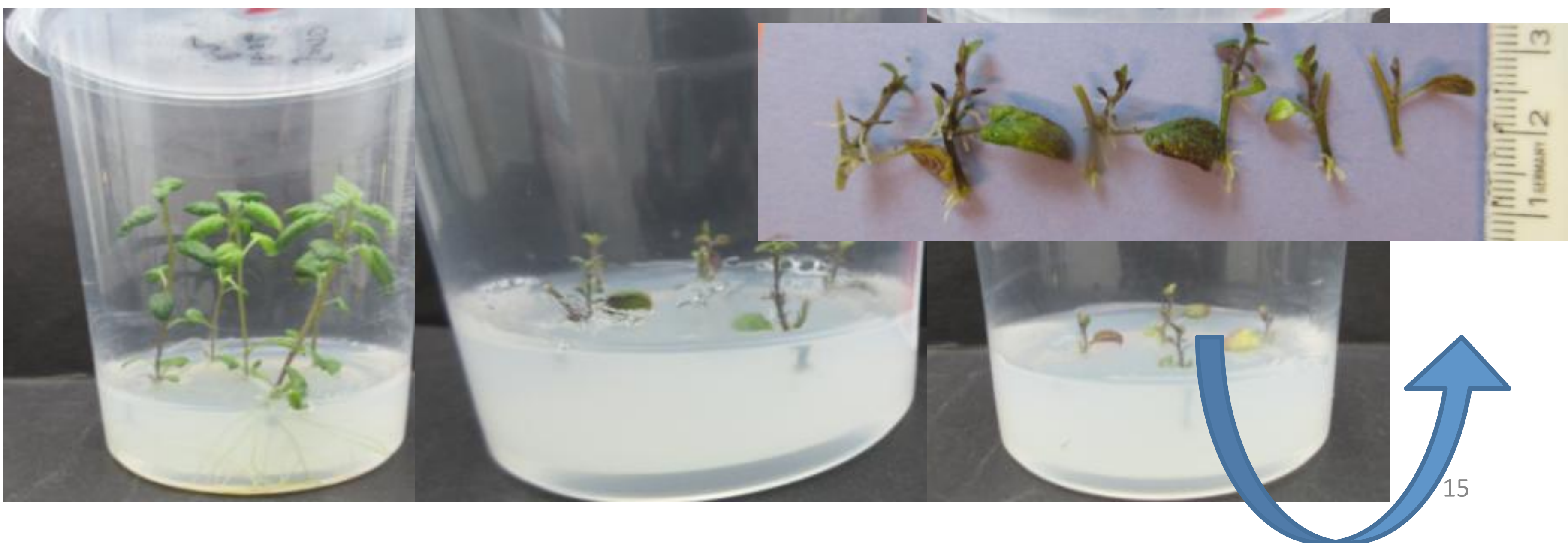
- Where performed using different statistical soft wares
- Results are presented in Tables and graphs

Results and discussion

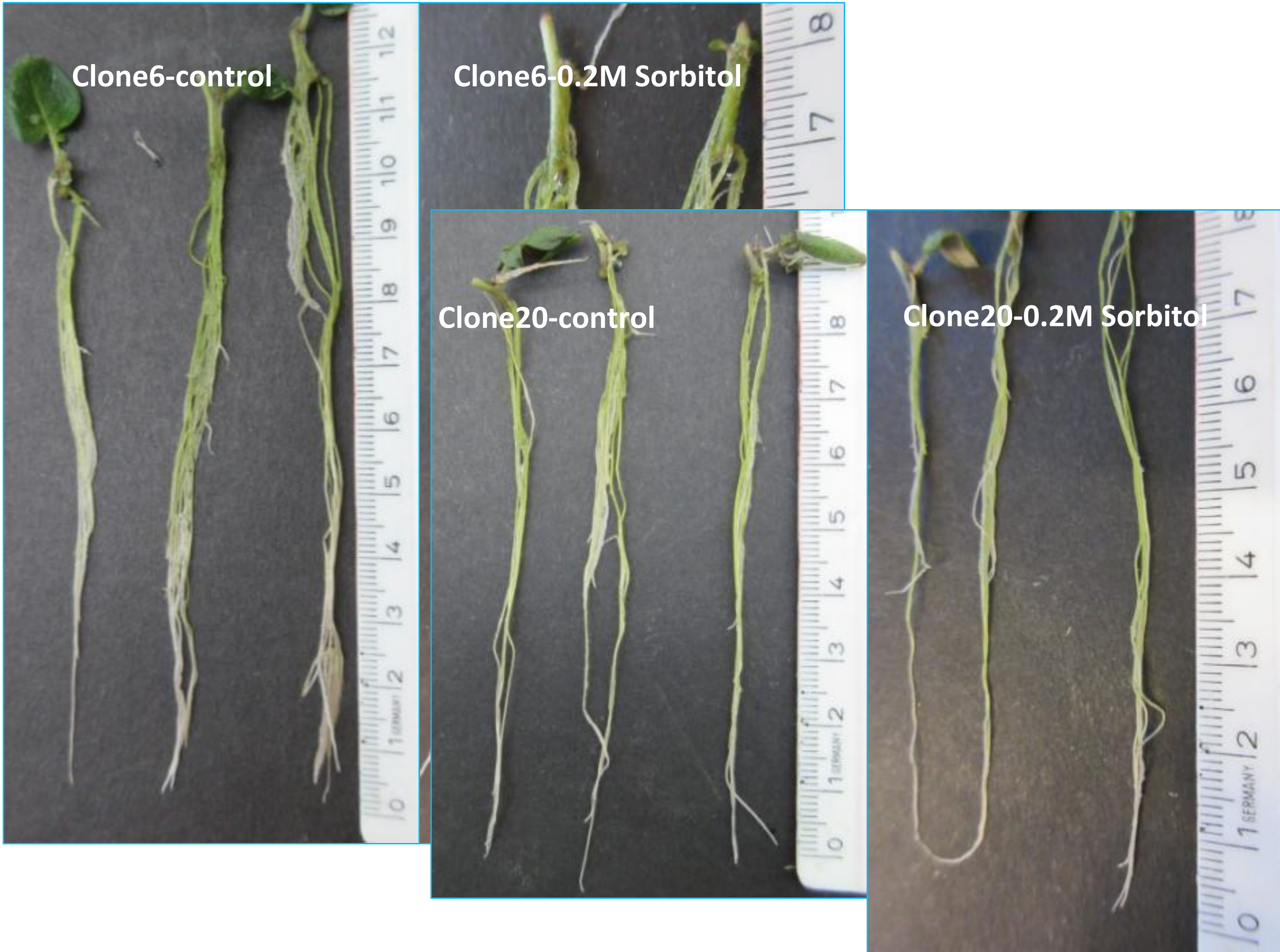
Growth performance of some potato genotypes under normal and water stress conditions








Clone31
Clone21
Challa
Bubu


Clone6-control
Clone6-0.2M Sorbitol
Clone20-control
Clone20-0.2M Sorbitol


Clone17-control


Clone14-control

Table 5. Distribution of morphological growth traits (mean±SD) of potato genotypes grown *in vitro* under non-stress and stress-environments.

Sorbitol treatment		Days to root initiation	Days to shoot initiation	Leaf number	Shoot height (cm)	Root length (cm)	Root number	Shoot DW (mg^{-1} plantlet)	Root DW (mg^{-1} plantlet)	R:S (DW)
	Mean±SD	8.8±1.5	8.6±1.3	6.3±0.8	4.1±1.3	8.6±1.9	11.1±3.7	4.9±2.2	0.4±0.2	7.2±2.7
Control	Minimum	5.0	5.0	4.17	1.58	2.90	4.76	0.15	0.10	2.40
	Maximum	14.0	13.0	8.80	8.18	12.70	27.45	15.56	0.92	15.50
	Mean±SD	11.1±2.17	10.9±1.7	5.7±0.1	2.9±1.1	6.9±1.7	9.3±3.2	4.3±2.3	0.5±0.2	7.6±2.5
0.1 M	Minimum	7.0	7.0	2.00	1.00	1.22	1.45	0.06	0.11	1.50
	Maximum	24.0	15.0	8.70	7.34	12.58	18.84	15.14	1.26	13.83
0.2 M	Mean±SD	14.4±2.8	14.5±2.7	4.5±1.4	1.6±0.7	5.4±1.6	5.4±2.2	2.8±1.7	0.5±0.3	5.8±2.4
	Minimum	8.0	8.0	1.00	0.20	0.45	1.05	0.04	0.15	1.23
	Maximum	22.0	25.0	7.60	4.57	9.64	13.82	9.12	1.76	11.60

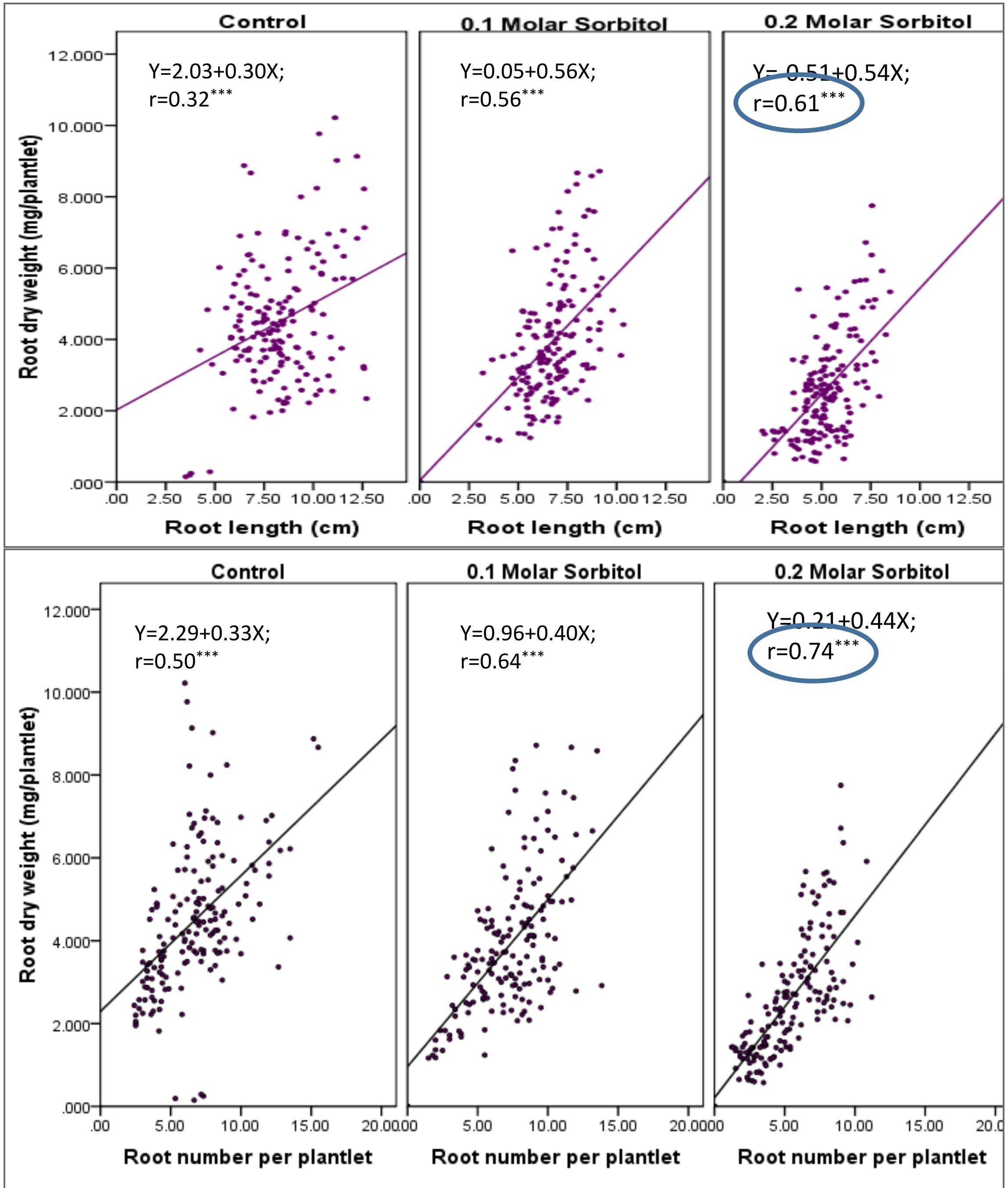
SD-standard deviation; R:S-root to shoot ratio; DW-dry weight

Table 6. Pearson correlation coefficients between traits under normal and Sorbitol induced water stress

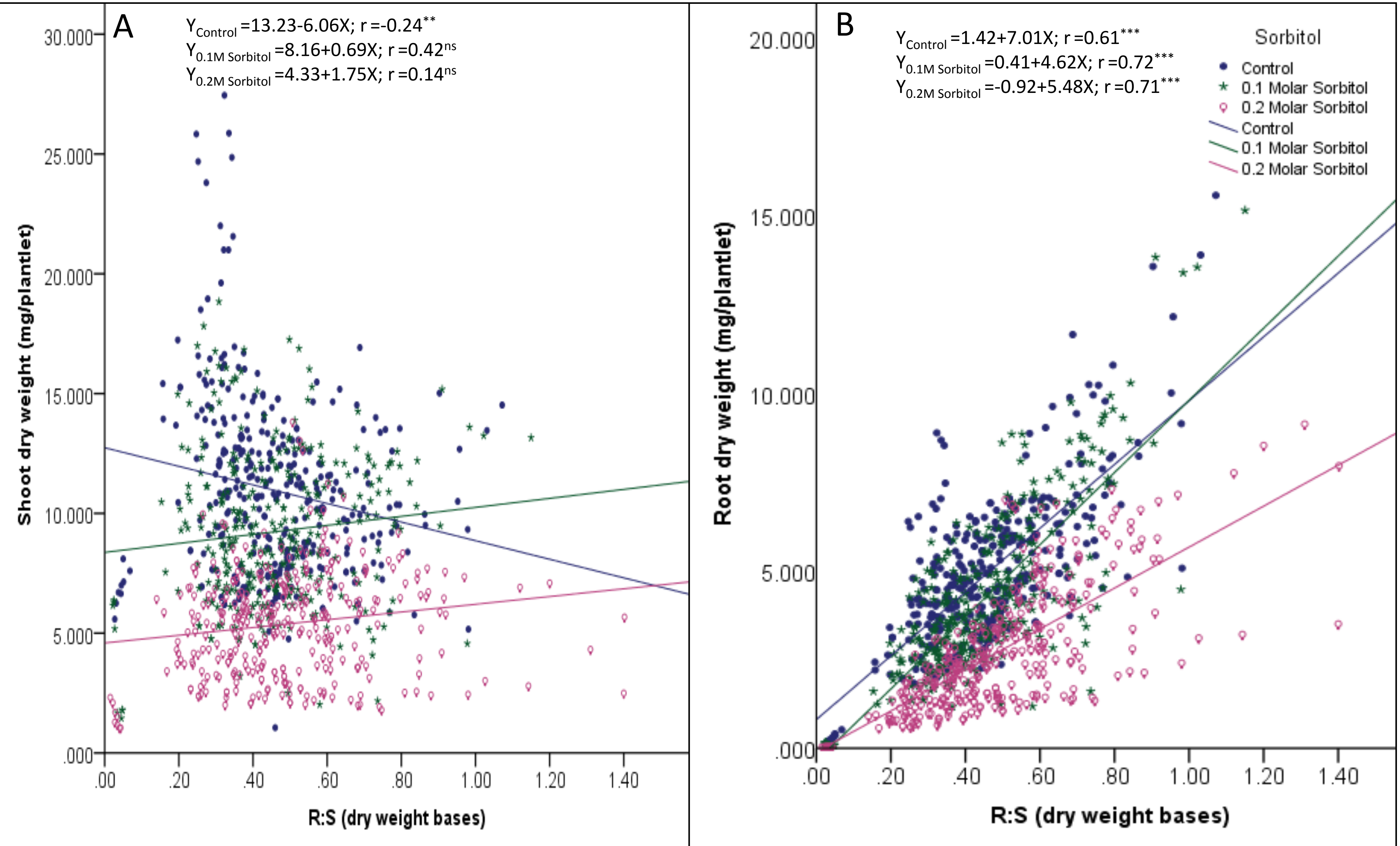
Treatments	Traits	Leaf number	Shoot height	Shoot fresh weight	Root fresh weight	Root length	Root number	Shoot dry weight	Root dry weight	R:S (fresh weight)	R:S (dry weight)
		Moisture stress induced by 0.2M Sorbitol									
Control	Leaf number	0.407***	0.214**	0.367***	0.151^{ns}	0.099ns	0.340***	0.398***	0.238**	-0.133^{ns}	-0.106^{ns}
	Shoot height	0.294***	0.527***	0.329***	0.239**	0.218**	0.238**	0.299***	0.196*	-0.008^{ns}	-0.039^{ns}
	Shoot fresh weight	0.200**	0.270***	0.401***	0.113^{ns}	0.148^{ns}	0.235**	0.340***	0.098^{ns}	-0.132^{ns}	-0.140^{ns}
	Root fresh weight	**0.601*****	**0.614*****	**0.612*****	**0.614*****	**0.515*****	**0.545*****	**0.529*****	**0.581*****	**0.306*****	**0.336*****
	Root length	0.280***	0.182*	0.189*	0.300***	**0.497*****	0.202**	0.196*	**0.371*****	**0.260****	**0.389*****
	Root number	**0.371*****	**0.328*****	**0.493*****	0.245**	0.166*	**0.522*****	**0.482*****	0.238**	-0.043^{ns}	-0.100^{ns}
	Shoot dry weight	0.245**	0.280***	**0.429*****	0.132^{ns}	0.196*	0.317***	0.447***	0.167*	-0.123ns	-0.145^{ns}
	Root dry weight	**0.573*****	**0.551*****	**0.599*****	**0.570*****	**0.550*****	**0.578*****	**0.621*****	**0.655*****	**0.315*****	**0.397*****
	R:S (fresh weight)	0.323***	0.303***	0.099^{ns}	0.389***	0.386***	0.185*	0.079ns	0.373***	0.430***	**0.457*****
	R:S (dry weight)	0.406***	0.387***	0.265***	0.491***	0.497***	0.339***	0.252**	**0.532*****	**0.502*****	**0.610*****

Treatments	Traits	Leaf number	Shoot height	Shoot fresh weight	Root fresh weight	Root length	Root number	Shoot dry weight	Root dry weight	R:S (fresh weight)	R:S (dry weight)
		Moisture stress induced by 0.2M Sorbitol									
e stress induced by 0.1M Sorbitol	Leaf number	**0.638*****	**0.510*****	**0.617*****	0.352***	0.380***	**0.510*****	**0.538*****	**0.424*****	-0.129^{ns}	-0.007^{ns}
	Shoot height	**0.519*****	**0.766*****	**0.527*****	**0.403*****	**0.433*****	**0.413*****	**0.440*****	**0.440*****	-0.006^{ns}	0.146^{ns}
	Shoot fresh weight	**0.504*****	**0.477*****	**0.711*****	**0.450*****	**0.375*****	**0.551*****	**0.636*****	**0.511*****	-0.041^{ns}	0.068^{ns}
	Root fresh weight	**0.565*****	**0.532*****	**0.593*****	**0.762*****	**0.561*****	**0.565*****	**0.540*****	**0.747*****	0.408***	0.463***
	Root length	**0.325*****	**0.386*****	**0.284*****	**0.415*****	**0.583*****	**0.254****	**0.286*****	**0.377*****	0.237**	0.192*
	Root number	**0.467*****	**0.370*****	**0.582*****	**0.486*****	**0.341*****	**0.674*****	**0.619*****	**0.554*****	0.109^{ns}	0.120^{ns}
	Shoot dry weight	**0.509*****	**0.436*****	**0.640*****	**0.406*****	**0.452*****	**0.560*****	**0.680*****	**0.508*****	-0.048^{ns}	-0.012^{ns}

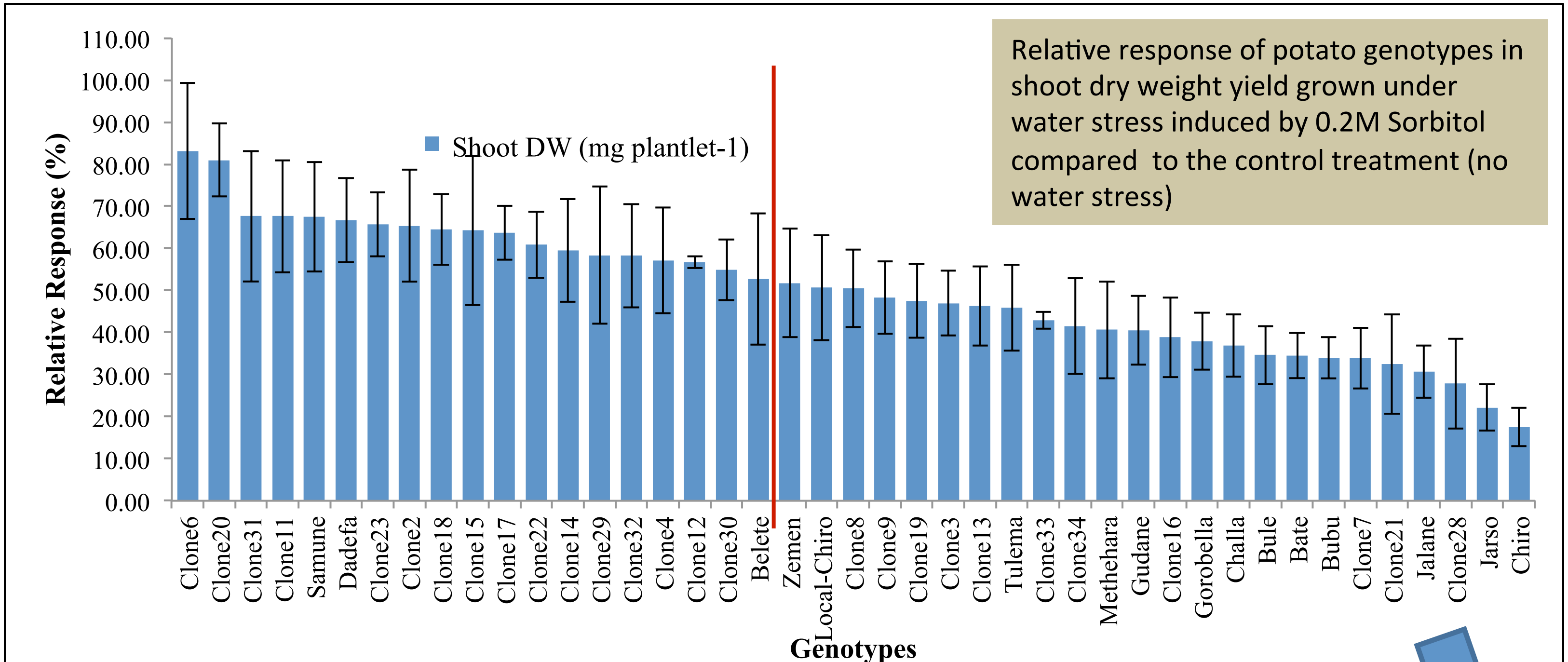
Trait(s) that have significant linear correlation in normal and water stress conditions could be stable and reliable indicator in the process of screening potato genotypes for drought tolerance

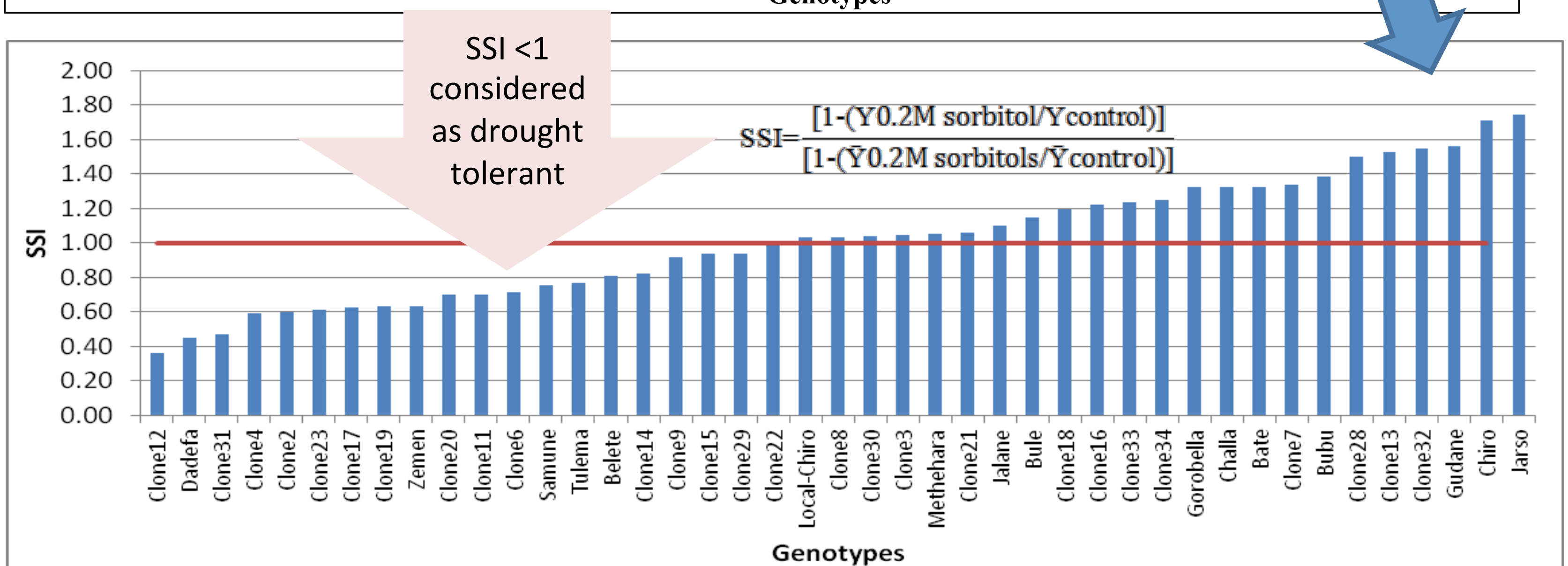


- **Root dry weight positively correlated with root length and root number**
- **When the plant experienced more drought stresses the association is more strong than under optimum conditions**
- **The trend is also the same for root fresh weight**



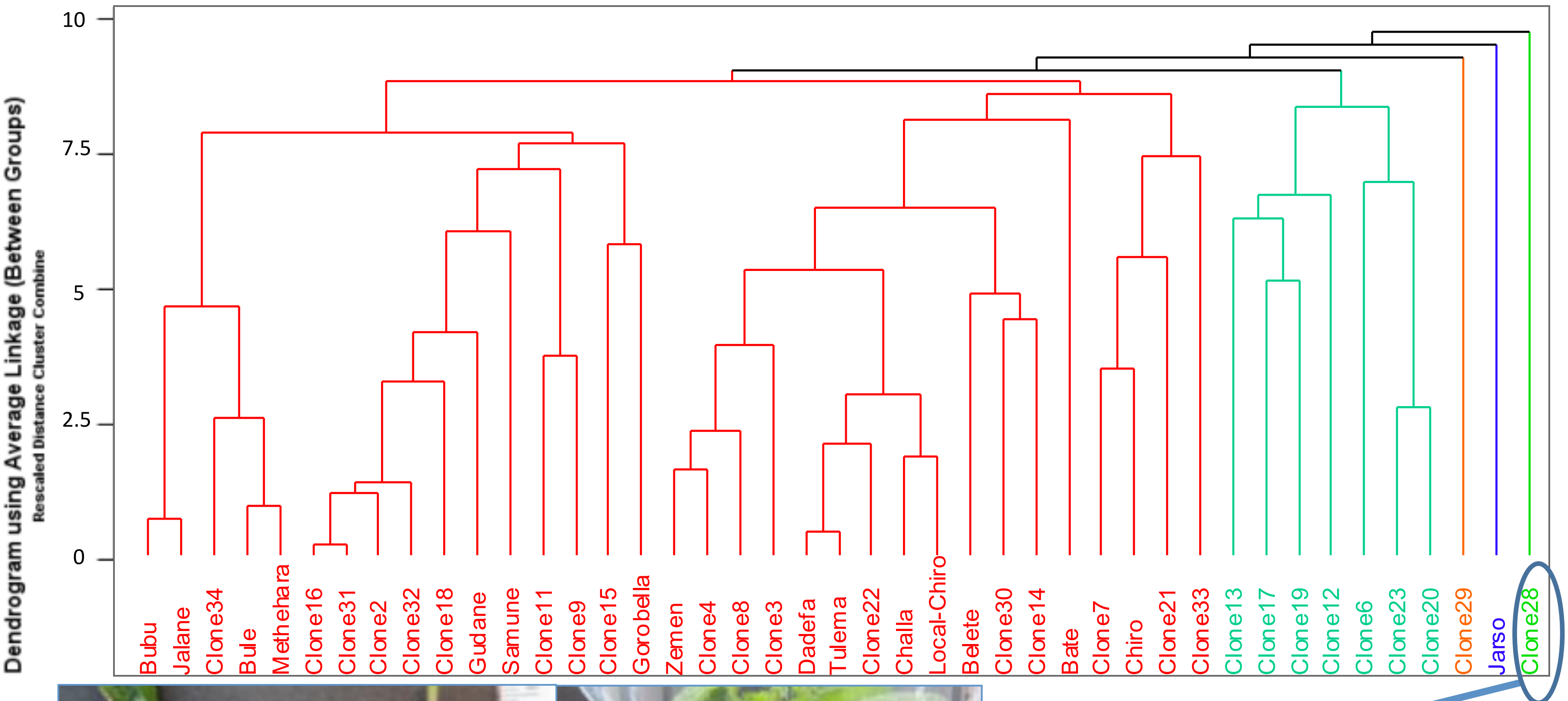
The increased partitioning of assimilate towards the root at the expense of the shoot (with negative correlations) could be an explanatory trait for the resistance to drought stress





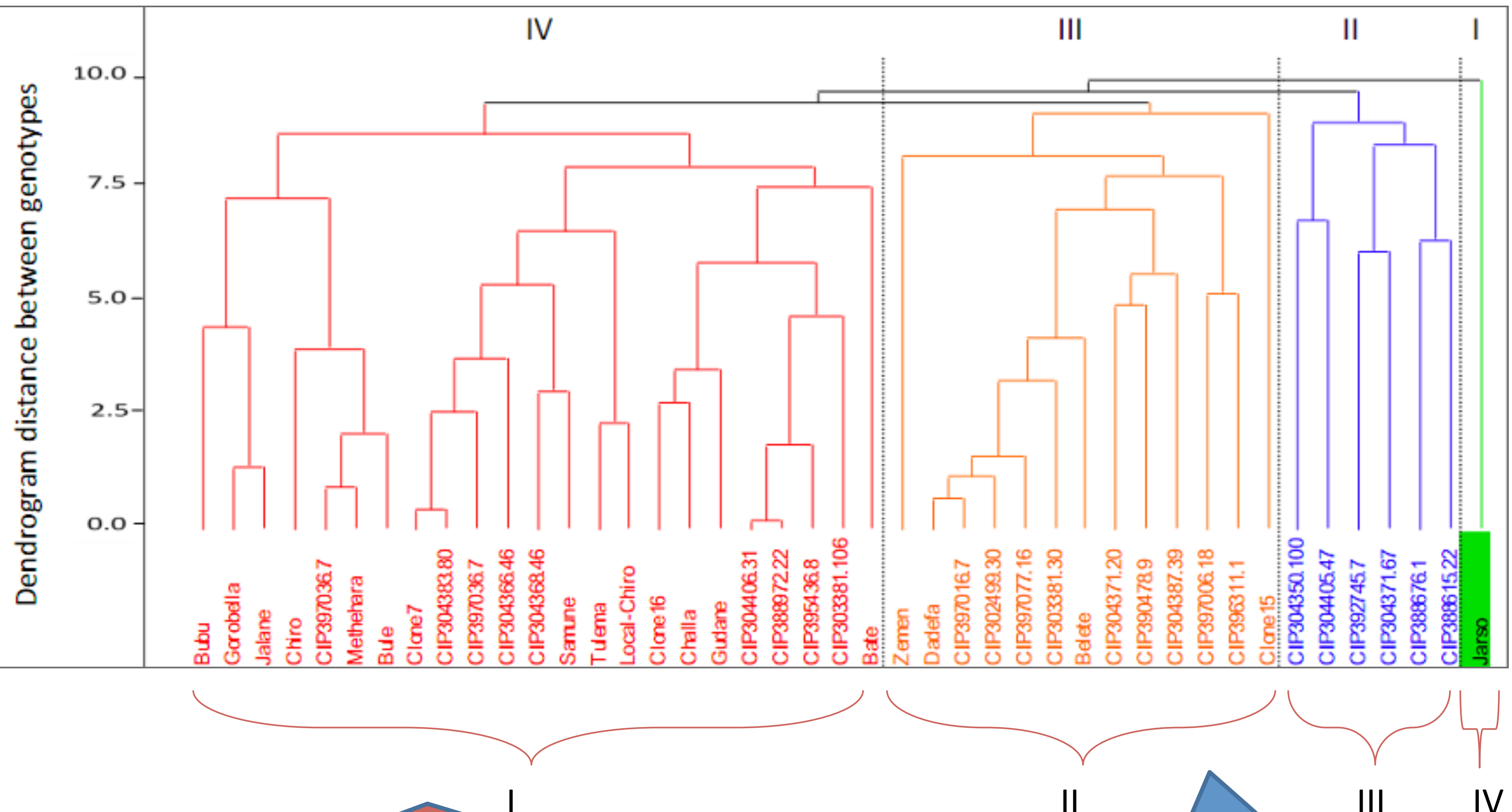
Stress susceptibility index (SSI) of 43 potato genotypes. Values are calculated from pooled mean of SSI values of all traits (days to root initiation; days to shoot initiation; leaf number (plantlet^{-1}); shoot height (cm plantlet^{-1}); shoot fresh weight (mg plantlet^{-1}); root fresh weight (mg plantlet^{-1}); root length (cm plantlet^{-1}); root number (plantlet^{-1}); shoot dry weight (mg plantlet^{-1}) and root dry weight (mg plantlet^{-1}).

Un weighted pairs of Hierarchical Clustering of 43 potato genotypes using 14 traits grown in vitro under no moisture stress conditions



Clustering of genotypes using 14 measured traits under non stress growth condition
Three genotypes outlier due to more vigorous growth or poor growth than others

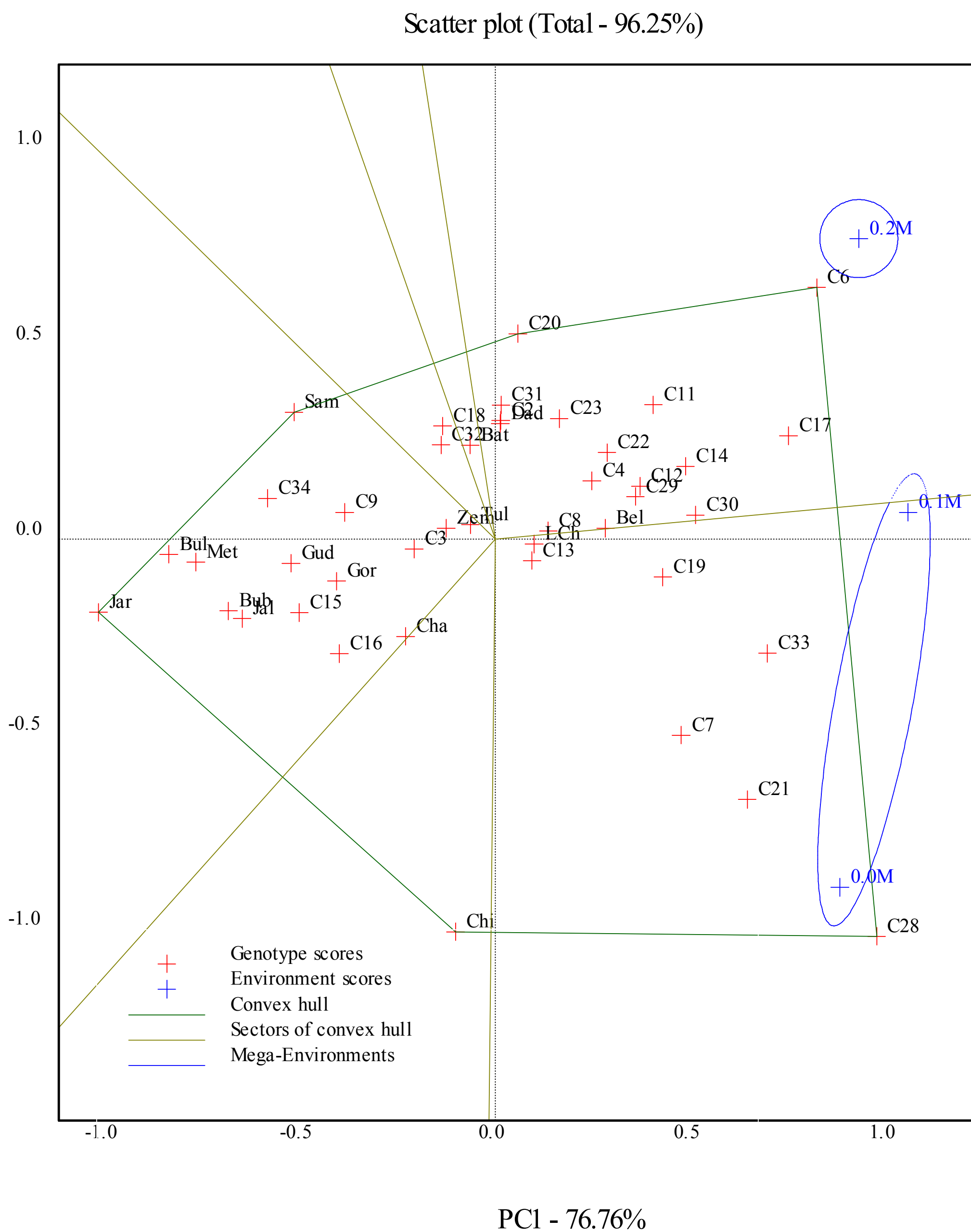
Un weighted pairs of Hierarchical clustering of 43 potato genotypes using 14 traits grown in vitro under moisture stress induced by 0.2M Sorbitol



Poorly performed genotypes

Clusters II and III are best performed genotypes

Poorly performed genotypes

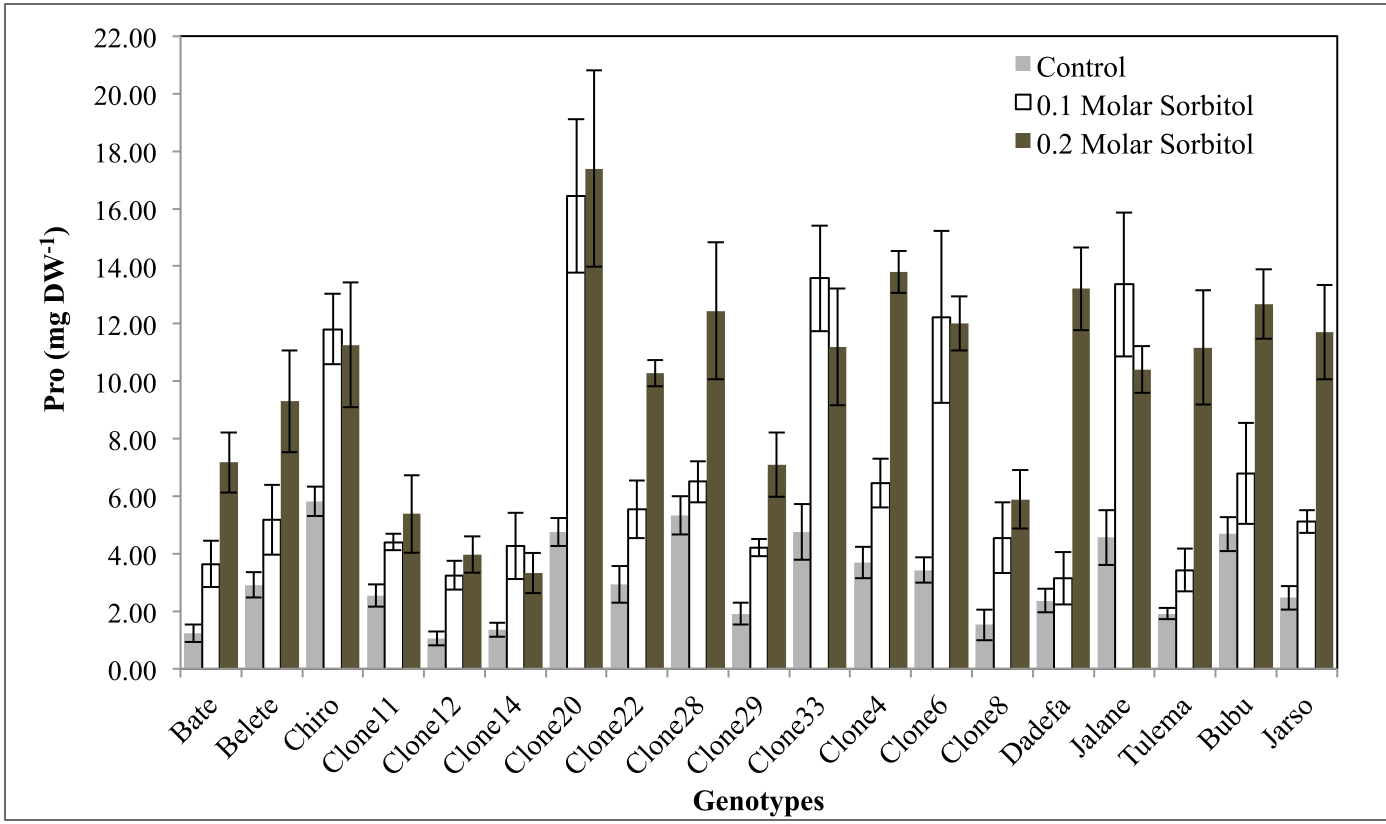


GGE biplot analysis showing the relation among environments (water stress induced by Sorbitol) on shoot dry weight (mg plant^{-1}) production of 43 potato genotypes

Tolerant (+PC1,+ PC2)	Moderately tolerant (+PC1, -PC2)	Moderately susceptible (-PC1, +PC2)	Susceptible (-PC1, -PC2)
Dadefa	Clone28	Tulema	Chiro
Clone20	Clone33	Clone2	Jarso
Clone31	Clone 7	Clone18	Bule
Clone12	Clone21	Samune	Challa
Clone11	Local Chiro	Clone34	Methera
Clone17	Clone8	Zemen	Clone 3
Clone6	Clone 13	Bate	Gudane
Clone29	Clone 19		Bubu
Clone4			Jalane
Clone19			Gorobella
Belete			Clone 15
Clone14			Clone 16
Clone 23			
Clone 22			

Kakani *et al.*; 2005; Uguru *et al.*; 2012

GGE biplot analysis showing the relation among environments (water stress induced by Sorbitol) on shoot dry weight (mg plant^{-1}) production of 42 potato genotypes



- All genotypes showed an increasing trend in proline accumulation regardless of their drought tolerance level
- Proline concentration increased by 1.22-3.56 folds at 0.1M Sorbitol compared to the control
  - The highest increment of more than three folds was obtained for the most drought tolerant clones (20, 6, 12 and 14)
- At 0.2M Sorbitol an increment ranges from 1.93-5.83 folds compared to the control
  - The highest increment of more than four folds was obtained for local cultivars (Jarso, Dadefa, Bate Tulema) and clone4 at sever stress drought

Conclusion

- Water stress induced by Sorbitol reduced the overall shoot growth of all evaluated potato genotypes in this experiment
- The traits are highly interacting among each other especially when the stress level is increased
- Different response mechanisms to water stress
  - Increased in root number and root total biomass
  - Reduced shoot height and leaf size
  - Increased in R:S especially in dry weight bases
  - Partitioning of assimilates toward root production in the expense of shoot growth
- Differential response of different potato genotypes in proline accumulation indicated variability at biochemical levels of the genotypes
- There are a promising **drought tolerant potato genotypes** in local cultivars, improved variety and CIP accessions
- These promising genotypes should be further evaluated under field conditions and validated for drought tolerance

Thank you

DANDENA GELMESA SOBOKA

- e-mail: dandenagalmesa@gmail.com
- Skype: dandena.gelmesa

Sponsors Of the World Congress on Root and Tuber crops

Thank you

WORLD CONGRESS ON ROOT AND TUBER CROPS

CATAS

GXAS

GSCRI

INTERNATIONAL SOCIETY FOR TROPICAL ROOT CROPS

BILL & MELINDA GATES foundation

GCP21

CIAT
International Center for Tropical Agriculture
Since 1967 Science to cultivate change

CENTRO INTERNACIONAL DE LA PAPA
CIP
A member of the CGIAR Consortium

CGIAR
RESEARCH PROGRAM ON Roots, Tubers and Bananas

IITA
Research to Nourish Africa

DFID Department for International Development

Ingredion

NEXTGEN CASSAVA

TTDI

CTA

Cibus

GWKJ

Perten INSTRUMENTS
a PerkinElmer company

oebiotech 欧易生物

SINO-FOOD MACHINERY

ROQUETTE