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Project :IAEA RAS/5070, Plant Mutation Breeding of Bio-Energy Crops for Optimizing Marginal Land Productivity

Project Team

1- Iftikhar Ali	**Plant Breeder**
2- Hafiz Munir Ahmed	**Plant Breeder**
3- Pervez Khan	**Soil scientist**
4- Zahid Ali	**Soil Scientist**
5- Mumtaz Ahmed	**Biochemist**

Mutation Breeding
Field Trials, 2016 & 2017

Crop: Rapeseed

Project Objectives

i- Optimization of mutagenesis for genetic improvement of Rapeseed as Bio-Energy Crop

ii- Identification, isolation and development of rapeseed mutant germplasm suitable for marginal / unproductive / water -deficit cultivation

Materials & Methods

Initial Parent : Rapeseed Mutant Variety ‘Abasin-95’

Mutagenesis: Gamma seed irradiation : 0.8, 1, 1.2 Kgy

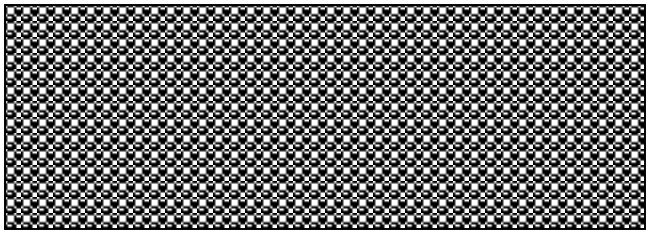
Development of Segregating Populations:
First Mutant Generation – M_1
Second Mutant Generation – M_2

Genetic Stability of M_3

Genetic Stability/Field Performance of M_4

Materials & Methods

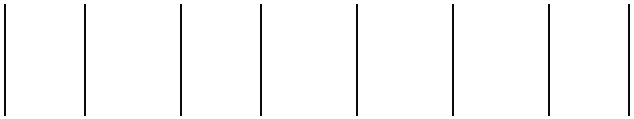
1st Year- M_1



Gamma Seed Irradiation

0.8, 1, 1.2 kgy

2nd Year- M_2

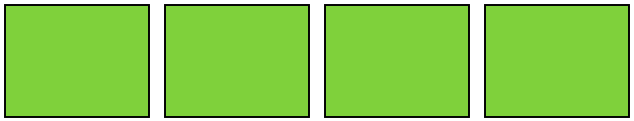


Segregating Populations

150 Single Plant Progeny Rows

3rd Year- M_3

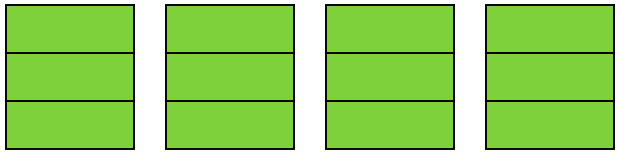
2015-16



Selection in superior heterogeneous progenies

4th Year- M_4

2016-17



Superior homogenous progenies bulked

Genetic stability of 14 promising mutant lines in agronomic field test

Field Experiments Mutation Breeding
Under IAEA RAS/05/070 at NIFA, Peshawar, Pakistan

M_4

M_3

M_2

M_1

Genetic Stability of M_3

Field Trials Data:

Agronomic Traits:

Plant Height, Branches/Plant, Pod Length, Seeds/Pod, Seed weight, Days to Flowering

Biochemical Traits:

Oil, Protein, Glucosinolates, Erucic, Oleic, Linolenic Acids

Statistical Analysis:

Analysis of Variance (ANOVA)
Analysis of Genetic Variability
Components of Co-Efficient of Variation – Genotypic, Phenotypic
Heritability, Genetic Advance

Genetic Stability of M_4

Genotypes: 14

Design:	**Randomized Complete Block**
Replications:	**4**
Plot size:	**$9m^2$**
Row Length:	**5m**
Row Distance:	**30cm**

Agronomic Traits:

Plant Height, Seed weight, Flowering, Seed Yield, Oil Yield

Biochemical Traits:

Oil, Protein, Glucosinolates, Erucic, Oleic, Linolenic Acids

Statistical Analysis:

Analysis of Variance (ANOVA)

RESULTS M_3

Estimates of mean values and genetic parameters for quality traits of M3

Treatment	Mean ±SE	Shift*	P value	CVp (%)**	CVg (%)†	h^2 (%)‡	Gs§
			Oil (%)				
Control	41.35						
0.8 Kgy	46.42	5.08	0.00	58.31	57.76	98.12	117.86
1 Kgy	45.75	4.41	0.00	51.27	50.55	97.22	102.68
1.2 Kgy	48.20	6.86	0.00	76.81	76.47	99.12	156.84
			Protein (%)				
Control	22.84						
0.8 Kgy	20.57	-2.27	0.00	52.98	52.28	97.35	106.26
1 Kgy	21.79	-1.05	0.01	25.01	23.54	88.59	45.65
1.2 Kgy	22.02	-0.82	0.05	20.52	18.32	79.73	33.70

Estimates of mean values and genetic parameters for quality traits of M3

Treatment	Mean ±SE	Shift*	P value	CVp (%)**	CVg (%)†	h^2 (%)‡	Gs§
Erucic Acid (%)							
Control	11.18						
0.8 Kgy	12.28	1.11	0.31	45.87	2.01	0.19	0.18
1 Kgy	12.74	1.57	0.09	75.34	64.93	74.27	115.26
1.2 Kgy	10.88	-0.29	0.74	41.02	12.12	8.73	7.38
Glucosinolate							
Control	20.98						
0.8 Kgy	25.24	4.26	0.11	108.09	91.74	72.02	160.38
1 Kgy	25.61	4.63	0.06	111.62	98.98	78.63	180.80
1.2 Kgy	19.39	-1.59	0.55	75.41	38.45	26.01	40.40

Estimates of mean values and genetic parameters for quality traits of M3

Treatment	Mean ±SE	Shift*	P value	CVp (%)**	CVg (%)†	h^2 (%)‡	Gs§
Linolenic Acid (%)							
Control	12.68						
0.8 Kgy	13.30	0.63	0.02	10.07	1.98	3.86	0.80
1 Kgy	13.25	0.58	0.03	24.03	21.94	83.35	41.27
1.2 Kgy	13.77	1.10	0.00	42.63	41.19	93.37	81.98
Oleic Acid (%)							
Control	52.88						
0.8 Kgy	52.22	-0.66	0.43	10.02	6.18	38.07	7.86
1 Kgy	53.37	0.49	0.44	7.39	4.52	37.36	5.69
1.2 Kgy	54.04	1.16	0.13	12.92	10.80	69.96	18.61

Estimates of mean values and genetic parameters for agronomic traits of M3

Treatment	Mean ±SE	Shift*	P value	CVp (%)**	CVg (%)†	h^2 (%)‡	Gs§
Number of Branches							
Control	12.04						
0.8 Kgy	12.12	0.09	0.70	9.22	3.17	11.79	2.24
1 Kgy	12.50	0.47	0.14	22.55	18.74	69.09	32.10
1.2 Kgy	12.26	0.23	0.57	18.11	8.86	23.94	8.93
Days to Flowering							
Control	58.78						
0.8 Kgy	55.48	-3.30	0.00	29.44	28.88	96.23	58.36
1 Kgy	55.34	-3.44	0.00	30.81	30.14	95.69	60.74
1.2 Kgy	54.86	-3.92	0.00	35.40	34.49	94.93	69.22

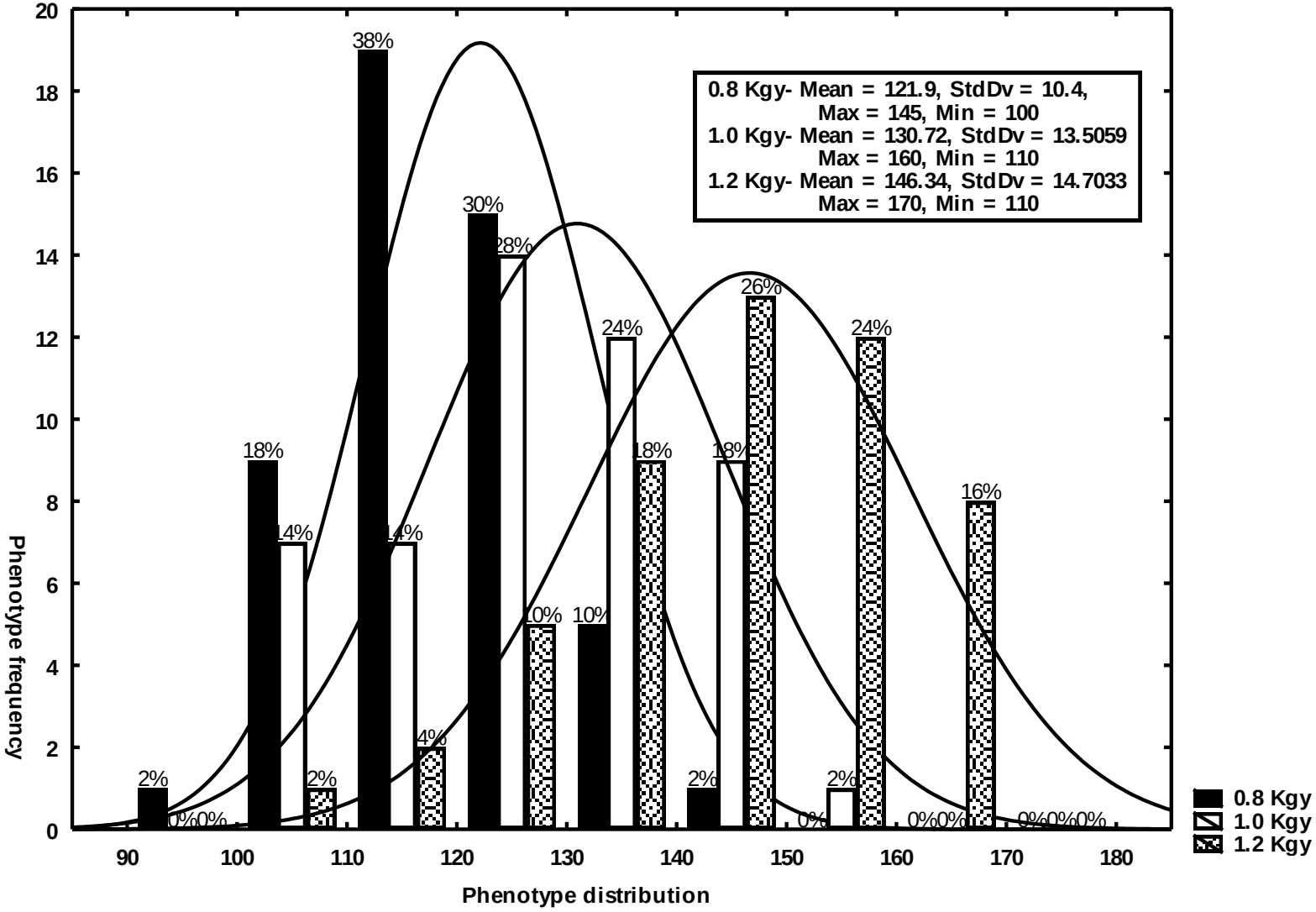
Estimates of mean values and genetic parameters for agronomic traits of M3

Treatment	Mean ±SE	Shift*	P value	CVp (%)**	CVg (%)†	h^2 (%)‡	Gs§
Plant Height (cm)							
Control	148.48						
0.8 Kgy	121.96	-26.51	0.00	98.27	98.04	99.54	201.51
1 Kgy	130.72	-17.75	0.00	64.84	63.57	96.13	128.39
1.2 Kgy	146.34	-2.13	0.59	14.93	7.00	22.00	6.76
Pod Length (cm)							
Control	5.41						
0.8 Kgy	6.72	1.31	0.00	107.90	107.78	99.79	221.80
1 Kgy	7.09	1.68	0.00	135.18	134.48	98.97	275.59
1.2 Kgy	6.72	1.31	0.00	109.22	108.32	98.36	221.31

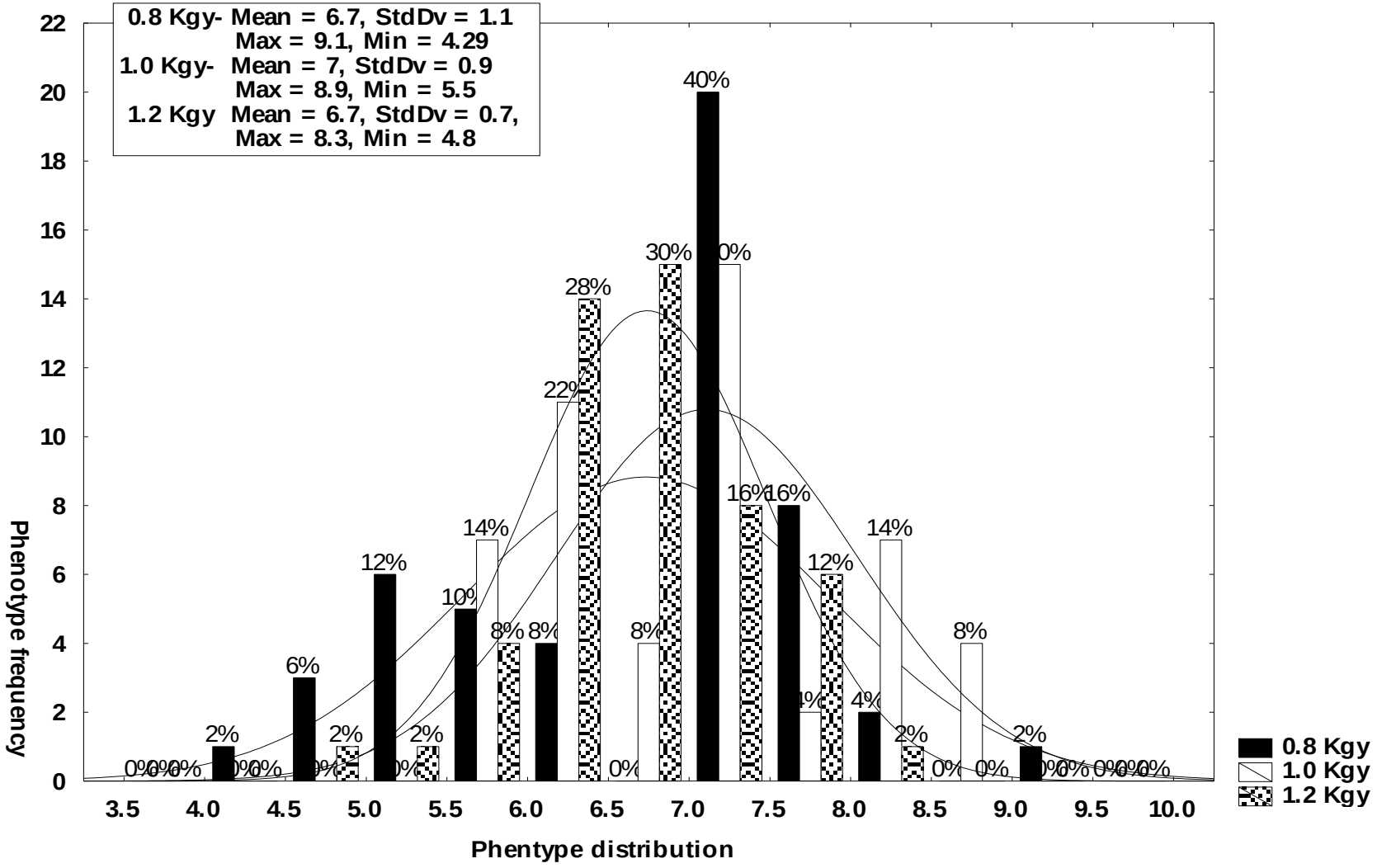
Estimates of mean values and genetic parameters for agronomic traits of M3

Treatment	Mean ±SE	Shift*	P value	CVp (%)**	CVg (%)†	h^2 (%)‡	Gs§
Seeds per Pod							
Control	20.48						
0.8 Kgy	21.17	0.69	0.00	16.89	16.61	96.73	33.65
1 Kgy	21.02	0.54	0.01	13.70	12.95	89.31	25.21
1.2 Kgy	20.94	0.46	0.00	11.67	11.10	90.37	21.73
Seed Weight							
Control	4.63						
0.8 Kgy	5.07	0.45	0.00	45.63	45.54	99.62	93.64
1 Kgy	5.44	0.82	0.00	81.84	80.51	96.78	163.15
1.2 Kgy	5.69	1.07	0.00	103.71	103.11	98.85	211.18

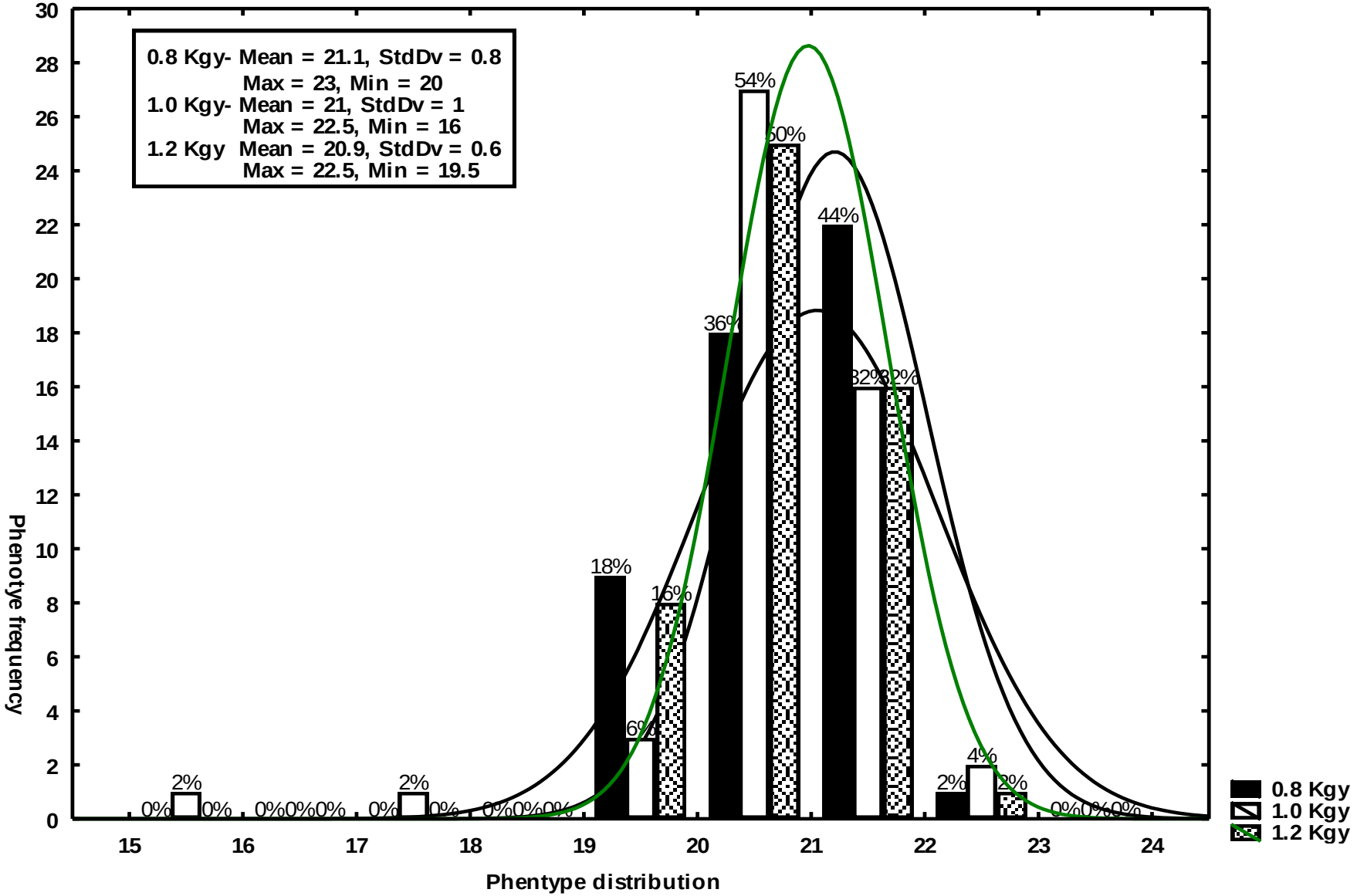
Progeny frequncy distribution for plant height (cm) in M_3 generation of rapeseed.



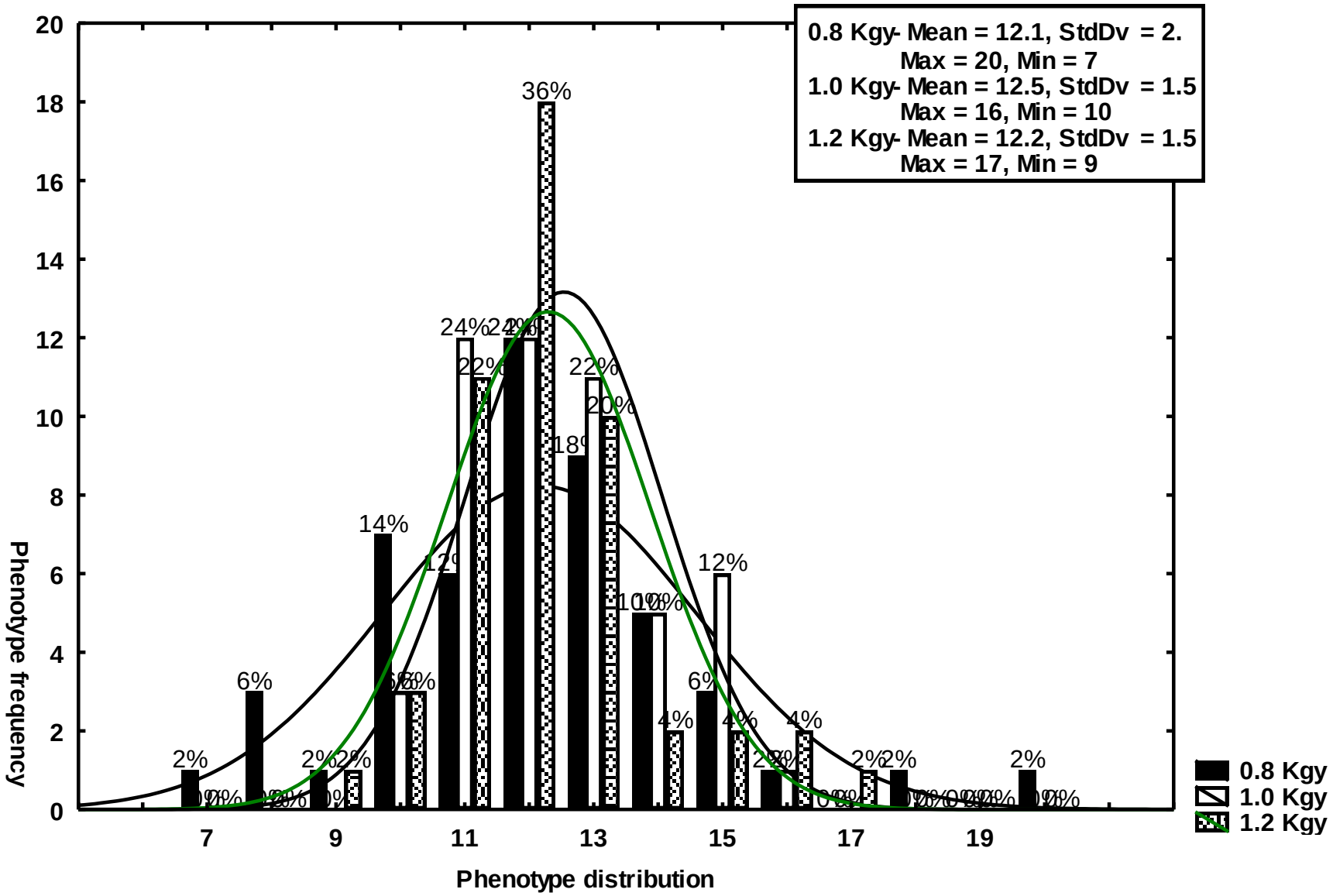
Progeny frequncy distribution for pod length (cm) in M_3 generation of rapeseed.



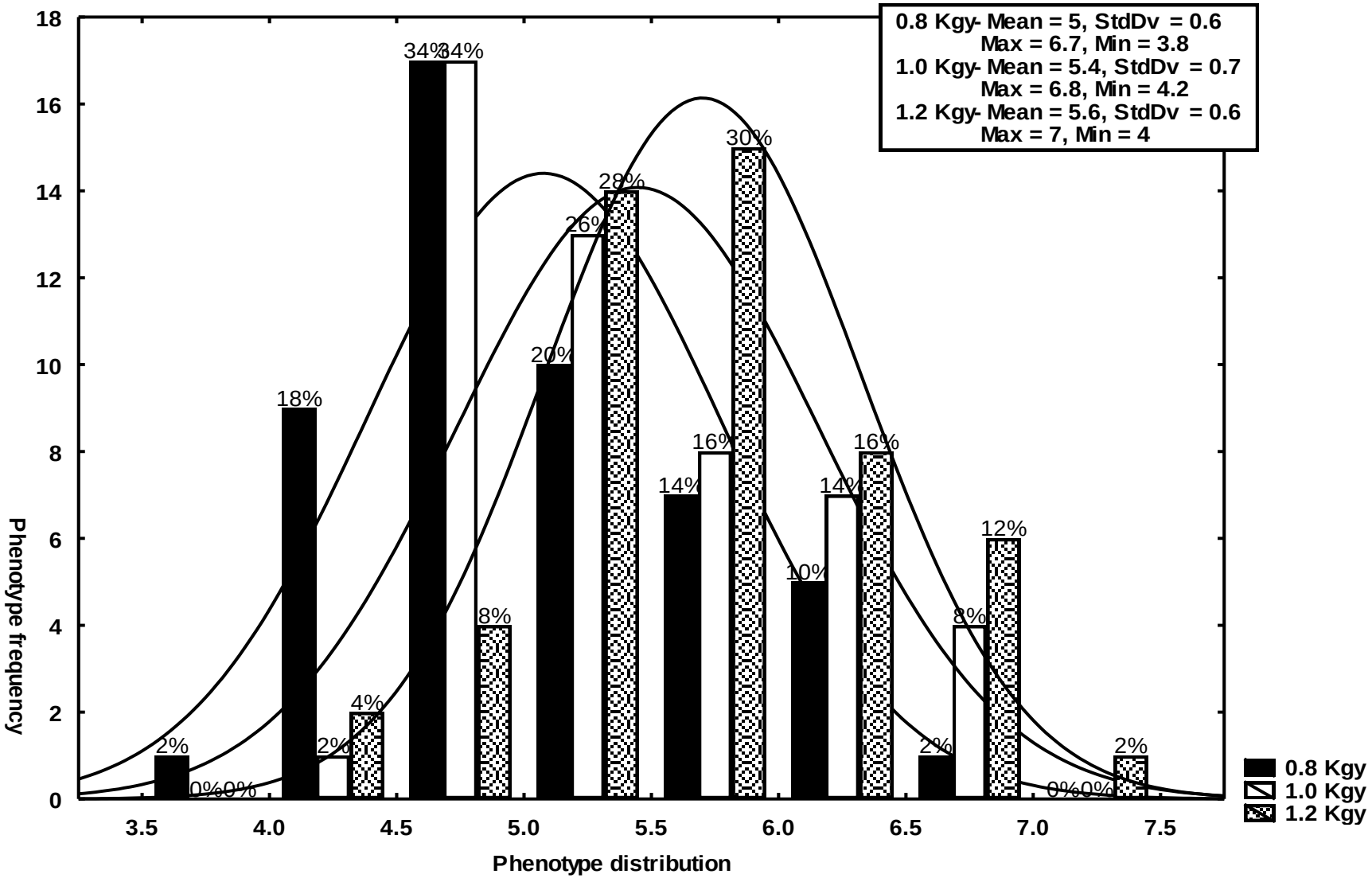
Progeny frequncy distribution for seed pod^{-1} in M_3 generation of rapeseed.



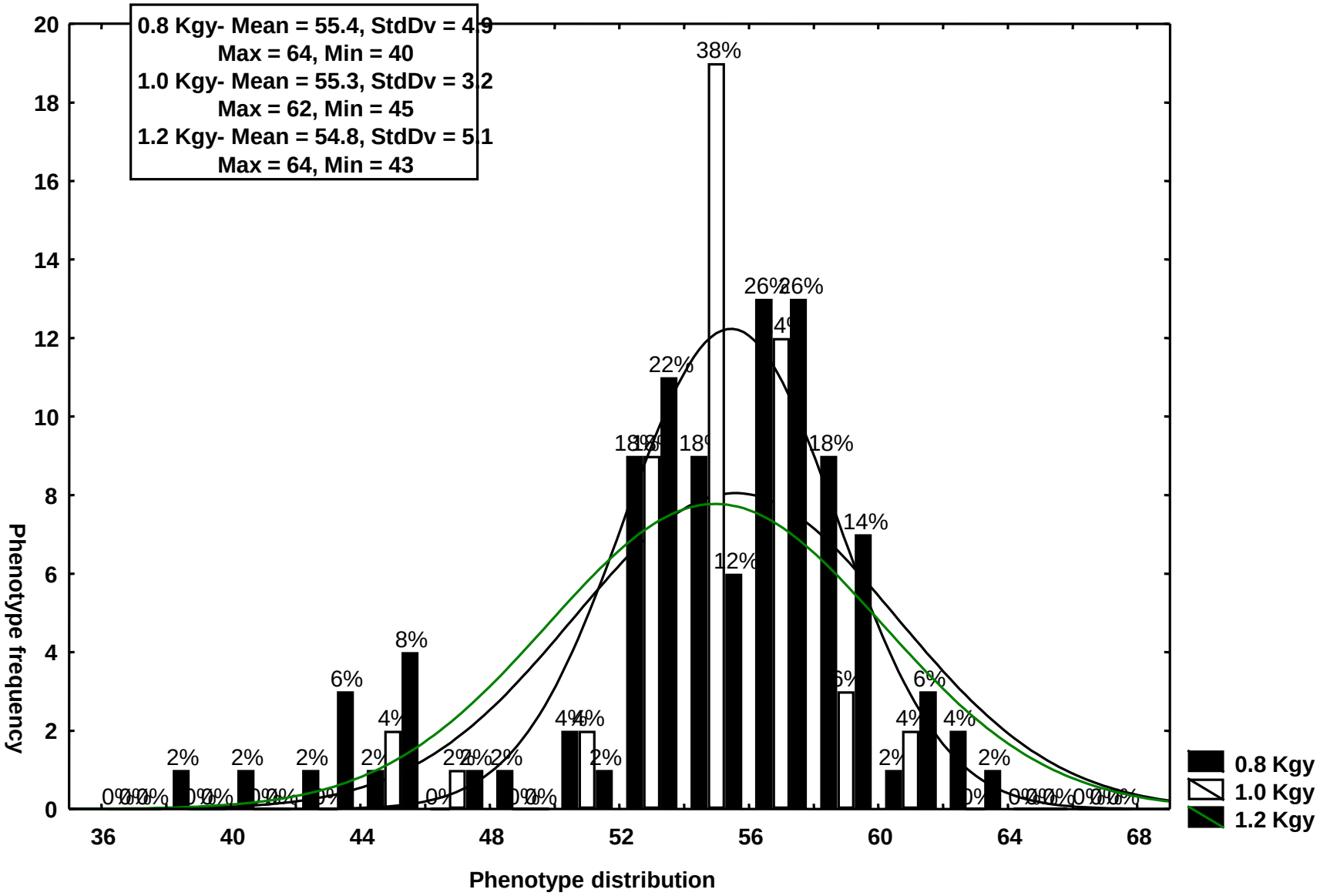
Progeny frequncy distribution for number of branches in M_3 generation of rapeseed.



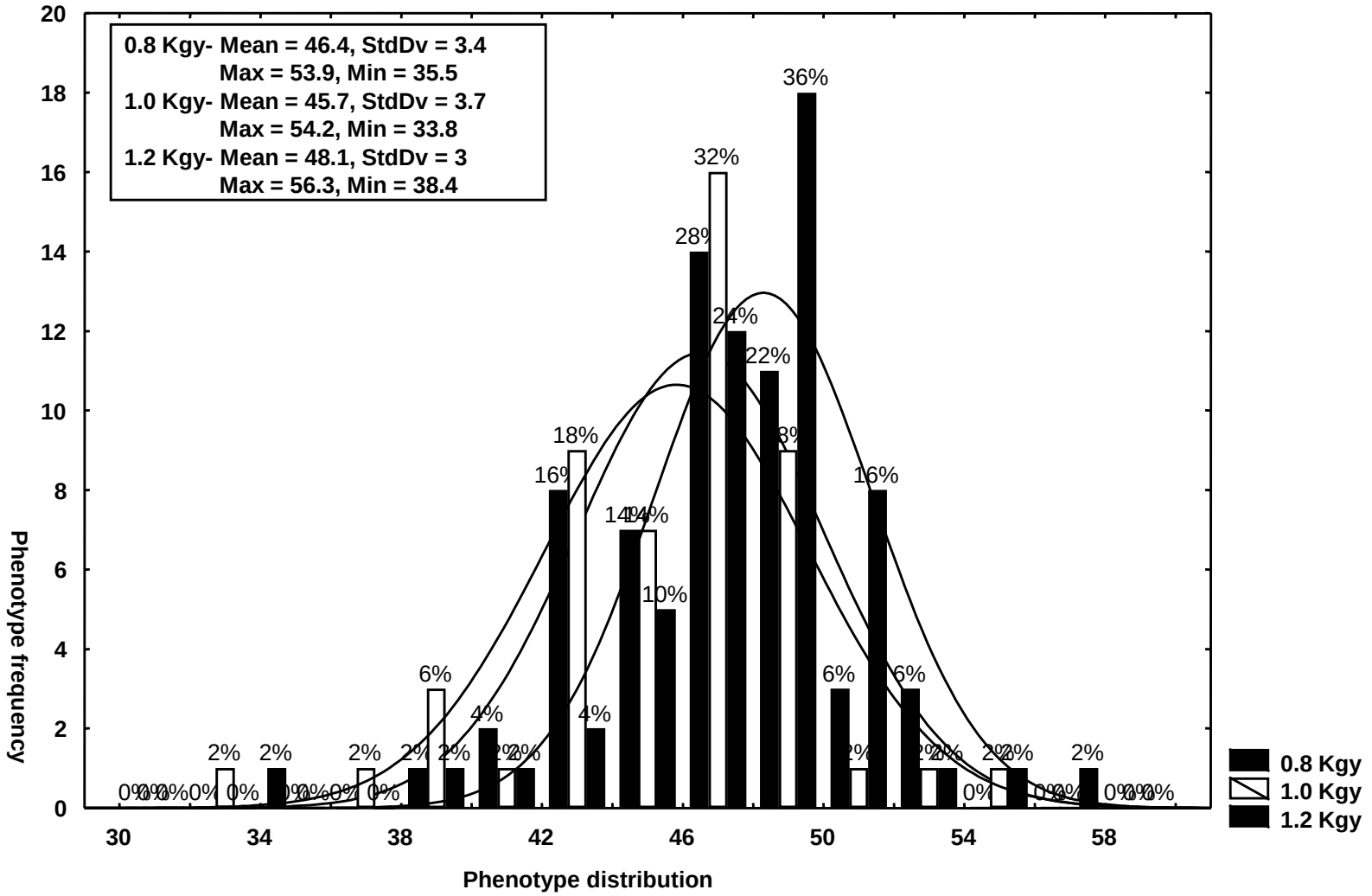
Progeny frequncy distribution for seed weight (g) in M_3 generation of rapeseed.

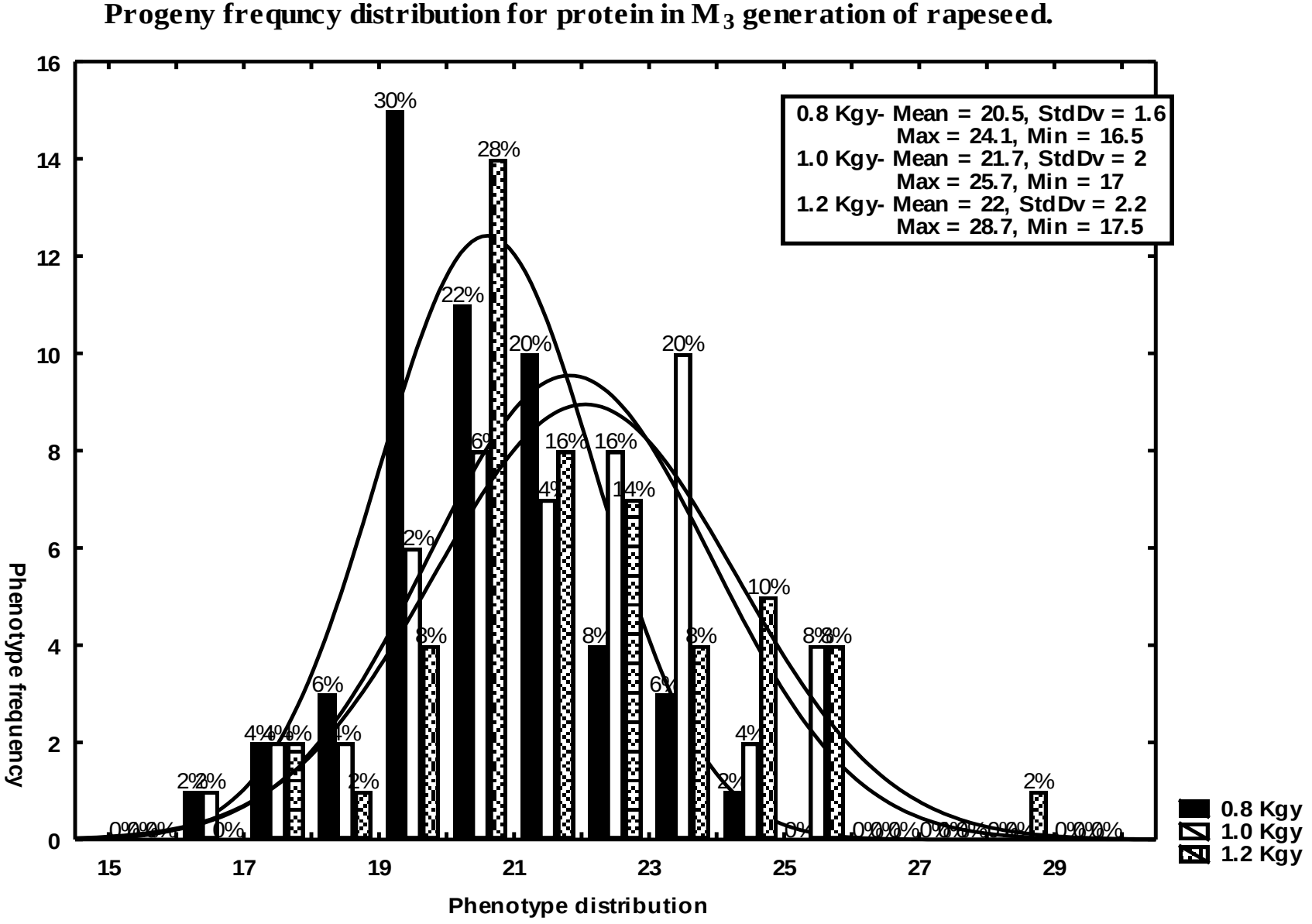


Progeny frequency distribution for days to flowering in M3 generation of rapeseed

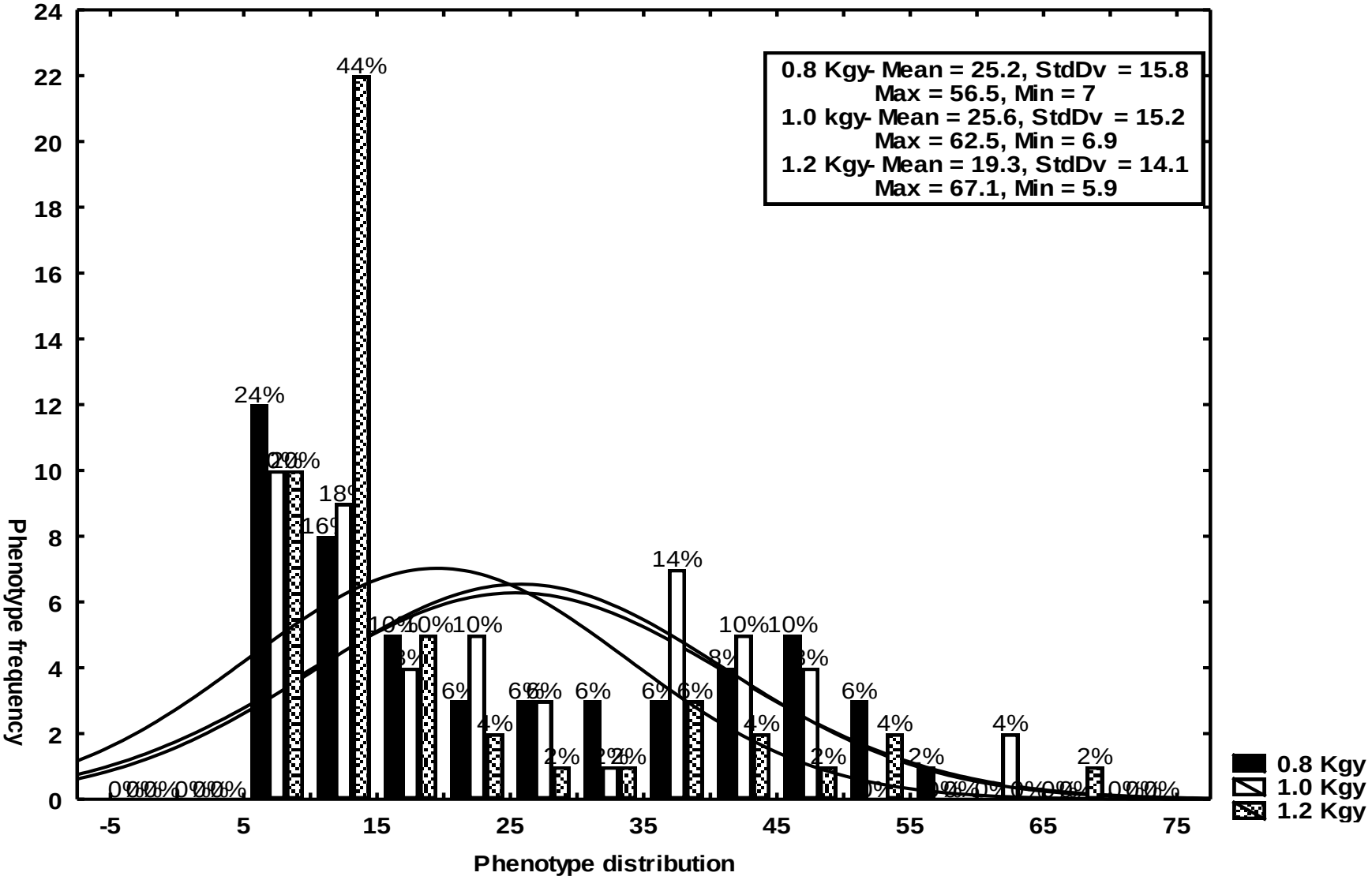


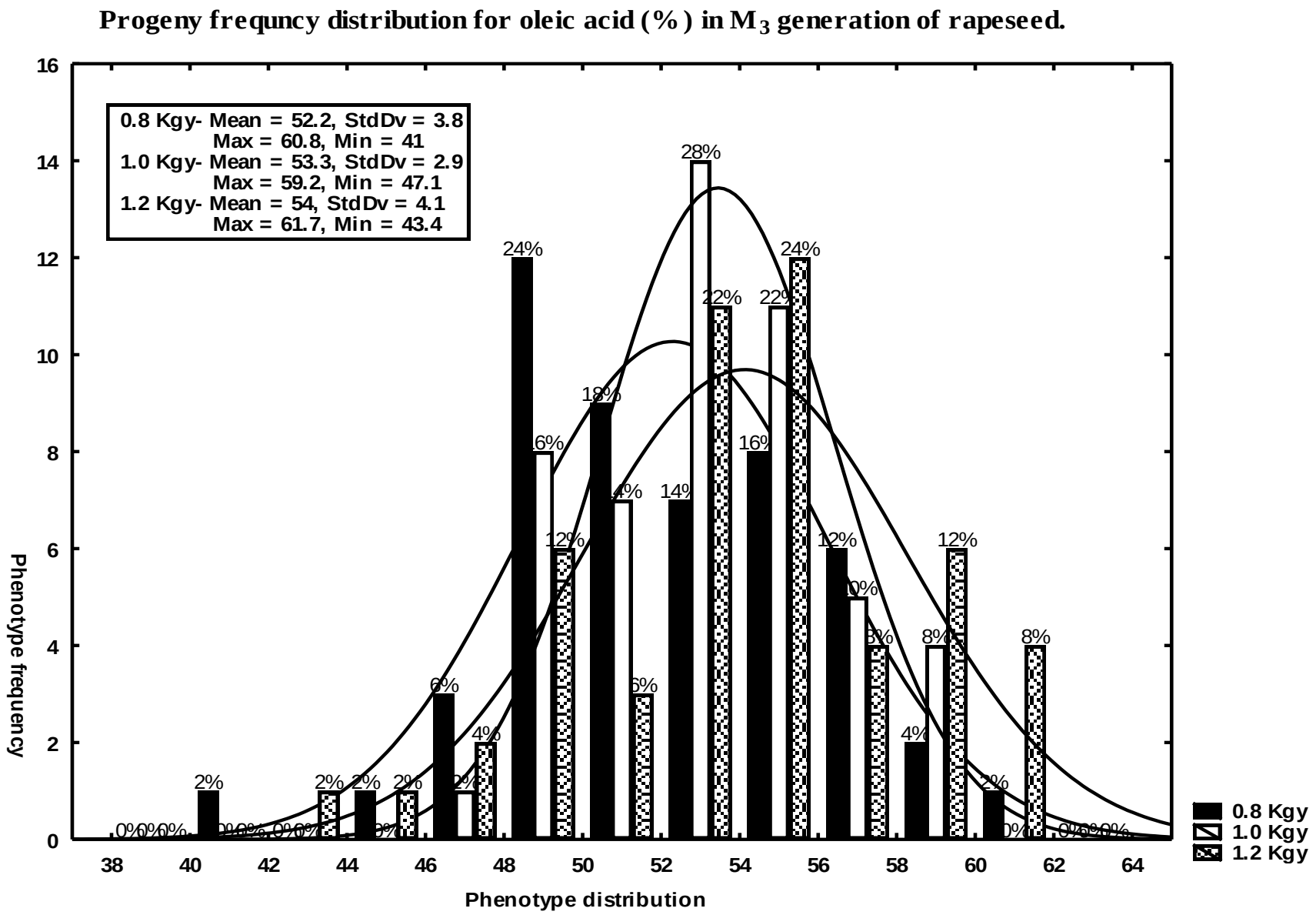
Progeny frequency distribution for oil content (%) in M3 generation of rapeseed



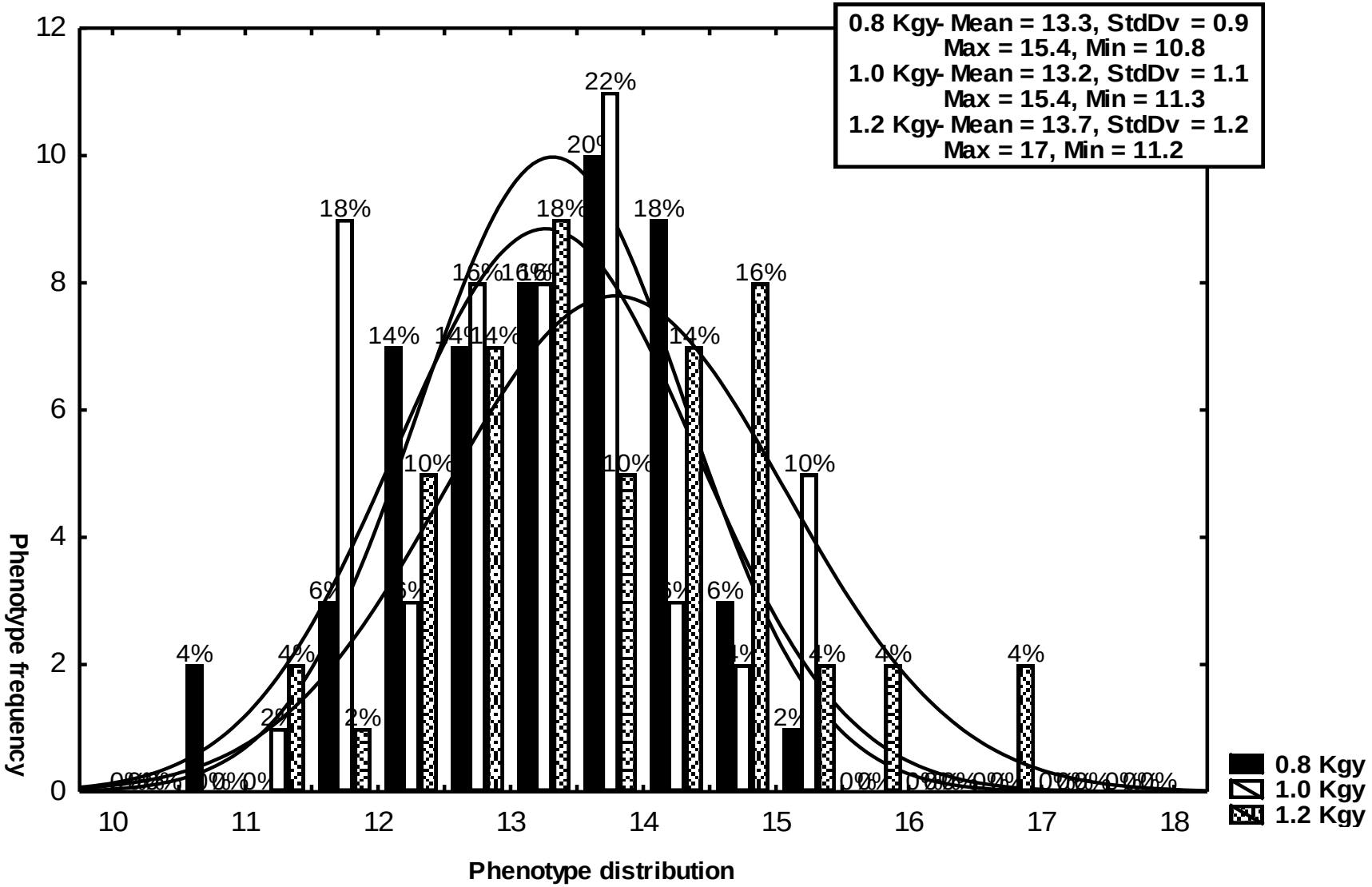

Progeny frequncy distribution for protein in M3 generation of rapeseed.
0.8 Kgy- Mean = 20.5, StdDv = 1.6
Max = 24.1, Min = 16.5
1.0 Kgy- Mean = 21.7, StdDv = 2
Max = 25.7, Min = 17
1.2 Kgy- Mean = 22, StdDv = 2.2
Max = 28.7, Min = 17.5
Phenotype frequency
Phenotype distribution
0.8 Kgy
1.0 Kgy
1.2 Kgy

Progeny frequncy distribution for glucosinolate (u mole g $^{-1}$) in M_3 generation of rapeseed.

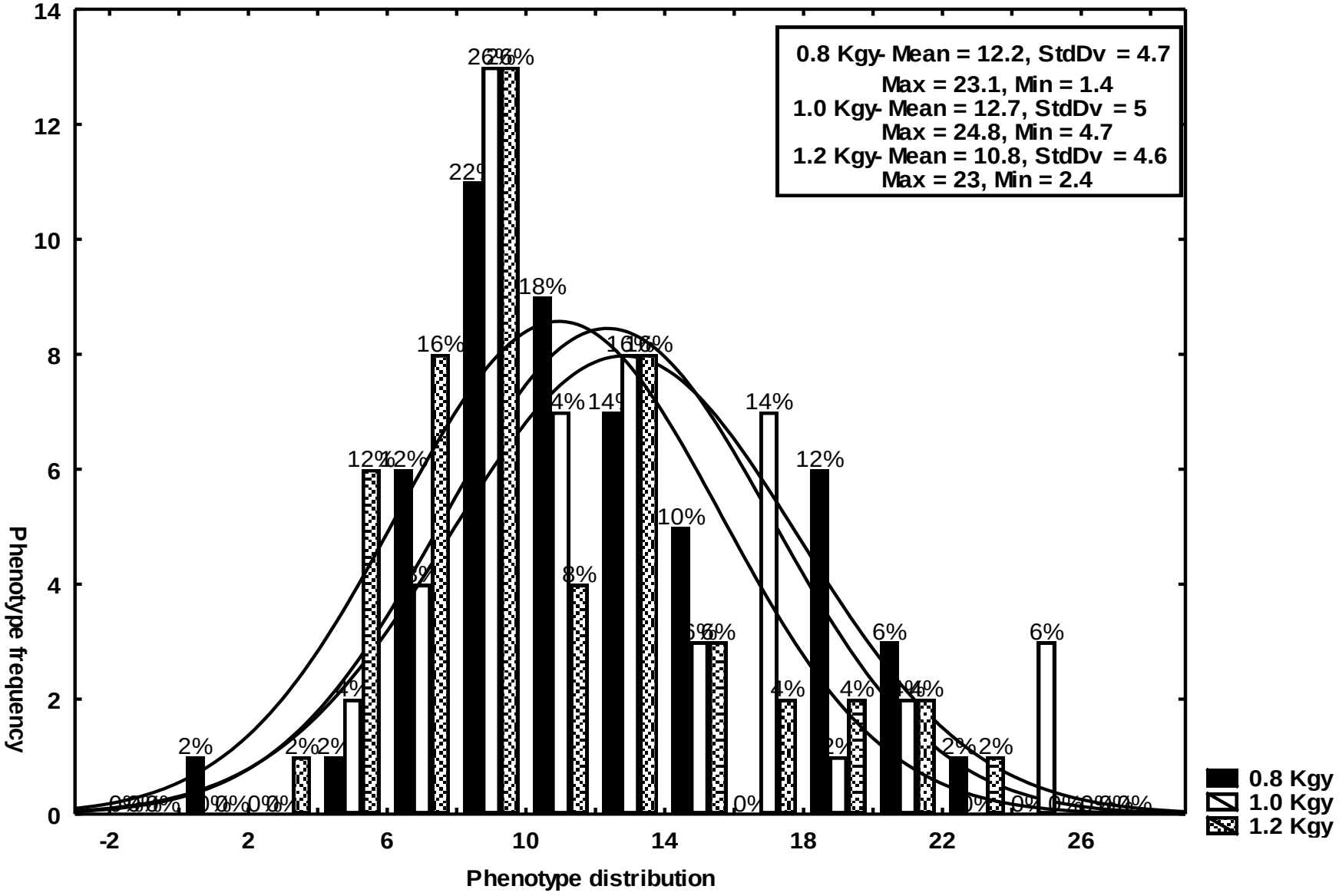



Progeny frequncy distribution for oleic acid (%) in M3 generation of rapeseed.
0.8 Kgy- Mean = 52.2, StdDv = 3.8
Max = 60.8, Min = 41
1.0 Kgy- Mean = 53.3, StdDv = 2.9
Max = 59.2, Min = 47.1
1.2 Kgy- Mean = 54, StdDv = 4.1
Max = 61.7, Min = 43.4
Phenotype frequency
Phenotype distribution
0.8 Kgy
1.0 Kgy
1.2 Kgy

Progeny frequncy distribution for linolenic acid (%) in M_3 generation of rapeseed.



Progeny frequncy distribution for erucic acid (%) in M_3 generation of rapeseed.



RESULTS M_4

Agronomic and yield components of rapeseed mutant lines (M_4 generation) in Preliminary Performance Trial

Mutant Lines	PHT	Flow	Mat	YD	OY	SW
PRML/01	103.33	72.33	159.67	2313.30	1092.50	3.37
PRML/02	115.33	131.33	212.67	2188.30	1026.80	4.53
PRML/03	95.33	85.67	162.00	2063.30	883.30	3.37
PRML/04	107.00	137.67	218.67	2186.70	970.80	4.70
PRML/05	94.33	68.67	150.00	2003.30	802.70	4.27
PRML/06	89.33	90.00	175.00	2030.00	824.40	3.87
PRML/07	111.33	93.67	180.67	2188.30	957.90	4.33
PRML/08	129.67	114.33	201.33	2100.00	882.30	4.30
PRML/09	102.00	86.00	173.00	2281.70	1043.40	3.77
PRML/10	109.00	119.33	206.33	2168.30	940.40	4.77
Abasin-95 (P)	122.67	93.33	183.00	1976.70	781.70	3.50
Mean	116.84	94.98	179.40	2138.30	926.03	4.15
F-test	**	**	**	**	**	**
C.V. (%)	7.06	3.08	2.25	2.40	7.06	8.82
LSD. 05	13.79	4.90	6.75	85.89	69.59	0.61
LSD. 01	18.60	6.61	9.10	115.87	93.89	0.83

Oil and seed quality components of rapeseed mutant lines (M_4 generation) in Preliminary Performance Trial

Mutant Lines	Oil	Protein	GSL	OA	LLA	EA
PRML/01	47.23	25.90	21.00	52.67	13.20	12.66
PRML/02	46.93	27.30	42.33	52.33	14.33	11.66
PRML/03	42.83	22.17	30.33	47.53	11.07	10.00
PRML/04	44.40	24.27	34.90	46.87	8.70	12.33
PRML/05	40.07	24.07	29.03	66.33	9.57	7.66
PRML/06	40.60	25.33	14.00	55.40	6.80	15.00
PRML/07	43.77	24.00	29.06	59.53	8.43	19.80
PRML/08	42.00	25.27	25.60	60.67	8.43	21.83
PRML/09	45.63	24.43	18.40	57.03	8.47	20.53
PRML/10	43.37	25.03	40.50	57.20	8.40	18.53
Abasin-95 (P)	41.60	24.77	23.46	54.50	8.70	20.16
Mean	43.00	24.75	27.00	55.77	9.34	17.00
F-test	**	**	**	**	**	**
C.V. (%)	2.64	4.02	8.22	4.84	6.12	8.22
LSD. 05	1.91	1.66	4.33	4.51	0.96	2.39
LSD. 01	2.58	2.24	5.85	6.08	1.29	3.22

Workshop on ‘Development of Bio-Energy Crops’ February 2, 2017 at NIFA, Peshawar, Pakistan

Atom News

An in-house Newsletter of Pakistan Atomic Energy Commission

January-February, 2017

NIFA Organized National Workshop on Development of Bio-Energy Crops

Pakistan is confronting persistent energy crisis while population growth and increasing consumption of power generation is not to enhance it exponentially. It needs a multifarious strategem including renewable energy resources such as hydro, wind, solar, and bio-energy. There has been considerable international attention on the expanded use of agricultural crops for the supply of bioenergy. The different challenges related to the production of feedstocks for bioenergy require integrated approaches. The modern agriculture has invaluable aspect knowledge in plant breeding, genetics and biotechnology which are considered to be crucial for future efficient bioenergy production. Plant breeding efforts at NIFA, Peshawar are underway to develop high yielding and better quality germplasm for the available arable crop production in the country.

Keeping in view the role of Plant breeding for the development of bioenergy crops in Pakistan, Plant Breeding and Genetics Division at NIFA, Peshawar organized one day workshop on "Development of Bioenergy Crops" on February 2, 2017. DR. Farhatullah, Meritorious professor The University of Agriculture, Peshawar, graced the occasion as Chief Guest. Speaking at the occasion, the Chief Guest highly acknowledged the PAEC and NIFA efforts for strengthening agricultural research and scientific culture in the Country. In welcome address, Dr. Aurang Zeb, Director-NIFA briefed the house about the action plan/achievements of PAEC and NIFA. In presentation lecture Dr. Iftikhar Ali., DCS/Organizer of the workshop, provided an overview of progress in mutation breeding of bio-energy crops.

Dr. Ehsan Ali, Director, Punjab Bioenergy Institute (PBI), Faisalabad, Professor Dr. Muhammad Akmal, Director, Climate Change Studies Centre, Peshawar, Professor Dr. Iqbal Munir, Director, Institute for Genetic Engineering & Biotechnology (IGEB), Peshawar, Professor Dr. Bashir Ahmad, and Assistant Professor Dr. Muhammad Arif, Department of Agronomy, The University of Agriculture, Peshawar did varied lectures highlighting the significance of Bio-energy crops and the status of opportunities and challenges for the development of bio-energy crops in Pakistan. In concluding session, Chief Guest awarded certificates to the participants and shields to the speakers of the workshop.

Results

Soil & Environment Sciences

Nuclear Institute for Food & Agriculture
Peshawar, Pakistan

Objectives

- **To assess rape seed genotypes response to nutrient and water through isotopic techniques**

- **To extend and demonstrate the efficacy of fertilization technology to farmers**

Physico-Chemical Properties of NIFA Experimental Field

Soil Texture	Silt loam
% Organic Matter	0.6
% N	0.035
Available P	7 ppm
K	100 ppm
pH	8.1
Ec	0.3 ds/m
% $CaCo_3$	19
Bulk density (g/cm^3)	1.34

Field Experiment

Crop	Rape Seed (RM1,RM2. RM3)
Nature of experiment	Field
Treatment	Four (4)
Replication	Three (3)
Plot size	3x 5m
Design	split plot design
Phosphorus and Potassium	@ 60 kg ha-1 Applied to all plots as a basal dose at sowing time
Nitrogen	➢@ 50,75,100 Kg ha^{-1} Applied in three splits (sowing , flowering, Pod formation)

Field Experiment

N fertilizer utilization	Three ^{15}N micro plots $1m^2$ in each Treatment to apply ^{15}N at sowing, flowering, pod formation stages (but due to unavailability not applied)
Water Use efficiency	Neutron access tubes down to 90cm in soil profile was installed in each treatment plots in two replications at the start of experiment. The probe was calibrated for the experiment site before start of the experiment. Neutron probe reading along with metrological observation was recorded regularly.
Date of sowing	October 10, 2016
Date of harvesting	April 13, 2017

Field Experiments Soil Science
Under IAEA RAS/05/070 at NIFA, Peshawar, Pakistan

PARAMETERS STUDIED

- **Crop Yield and Yield Components**
- **% N in Crop at different growth stage**
- **Water Use Efficiency**
- **Soil Analyses**
- **Meteorological Observations**

  (Rainfall, Temperature, Humidity)

Nitrogen concentration at Seedling, Flowering and Pod Formation Stages as affected by various Levels and Timings of N application

	N levels	% N at seedling stage	% N at flowering stage	% N at pod formation stage
Control		3.9 E	1.4 C	0.7 F
Rapeseed-M01	50	4.3 D	1.9 B	1.2 E
	75	4.9 C	1.9 B	1.4 D
	100	5.1 B	2.4 A	1.6 ABC
Rapeseed-M02	50	4.3 D	1.9 B	1.2 E
	75	4.9 C	2.1 B	1.5 CD
	100	5.2 AB	2.5 A	1.6 AB
Rapeseed-M03	50	4.5 D	1.9 B	1.2 E
	75	4.9 C	2.1 B	1.5 D
	100	5.3 A	2.4 A	1.7 ABC

N Concentration in Straw, Seeds, N uptake in seeds and N Use Efficiency as affected by various Levels and Timings of N Application

	N levels	% N in straw	% N in Grain	Grain N uptake kg /ha	Nitrogen use efficiency
Control		0.4 C	3.1 D	5.90 D	
RM1	50	0.5 AB	3.2 BC	8.47 B	5.15
	75	0.5 AB	3.3 AB	7.52 BC	2.16
	100	0.6 A	3.4 A	7.89 BC	1.20
RM2	50	0.4 C	3.2 BC	7.77 BC	3.75
	75	0.6 A	3.3 AB	7.00 C	1.47
	100	0.6 A	3.4 A	7.03 C	1.13
RM3	50	0.4 C	3.2 BC	8.16 B	4.53
	75	0.5 AB	3.3 AB	7.74 BC	2.45
	100	0.6 AB	3.4 A	11.63 A	5.73

Water Use Efficiency in Straw and Seeds as affected by various Levels and Timings of N Application

	N levels	Straw yield kg/ha	Straw WUE kg/ha/mm	Grain yield	Grain WUE kg/ha/mm
Control		874.0519	8.953615	193.3556	1.980696
RM1	50	1690.904	18.2406	264.6519	2.854928
	75	1482.393	11.88862	228.7185	1.834297
	100	1721.215	11.96119	234.3407	1.628497
RM2	50	1793.081	13.55827	243.9556	1.844654
	75	1659.452	13.59315	214.6222	1.758046
	100	1583.674	10.25563	208.9185	1.352924
RM3	50	1377.2	13.07137	252.4296	2.395877
	75	1391.215	15.96254	238.4148	2.735527
	100	1366.444	7.20015	344.6667	1.816138

Response of EBD

(Environmental Balance Device System)

Crop: Wheat ‘Variety Lalma’

Objectives:

- **To study the efficacy of EBD**

Experimental Details

Crop	Wheat Variety Lalma
Nature of experiment	Field
EBD	Two EBD stakes installed which is of green Top (plant growth)
Plot size	40x 40m
Phosphorus and Potassium	15 kg to 40*40m area = 62 kg/ha 5kg to 40*40m area =31 kg/ha
Nitrogen	18.5 kg to 40*40m area = 115kg/ha
Control plots	40 meters away from the EBD installed to one set of control same fertilizer level were applied and to other set fertilizer were applied at the rate of 150: 90:60 kg/ha NPK
harvesting	1 m^2 were at three locations from EBD at 2m, 13 m and 30 m

Biomass and wheat grain yield as affected by EBD

EBD	Biomass kg/m^2	Mean kg/m^2	Grain yield kg/m^2	Mean kg/m^2
2m from EBD stake	1.45, 1.48, 1.30	1.41 14 t/ha	0.375,0.360, 0.370	0.36 3.6 t/ha
13 m from EBD stake	1.32, 1.40, 1.36	1.36 13.6 t/ha	0.35,0.320, 0.34	0.33 3.3 t/ha
30 m from EBD stake	1.38, 1.30, 1.21	1.29 12.9 t/ha	0.31, 0.30, 0.35	0.32 3.2 t/ha
Control with same fertilizer level	1.14, 1,10, 1.15	1.13 11.3 t/ha	0.30, 0.29, 0.31	0.3 3 t/ha M_1
Control with recommended fertilizer level	1.49, 1.41, 1.45	1.45 14.5 t/ha	0.385, 0.349.0.389	0.37 3.7 t/ha

FUTURE WORK PLAN
2017 - 18

Work Plan: 2017-18

- **Induction of genetic variability , selection and evaluation of putative mutants will continue - M_1 /M_2 /M_3**

- **Selected high yielding M_4 Lines (7/10) will be evaluated for field performance under rain-fed environments in Multi-Location Trial to estimate the components of variance associated with interactions**

- **Stability assessment using stability procedures for selected (7/10) mutant lines**

- **Selected three (3) advanced mutant lines will be tested on marginal land for agronomic performance and other relevant Soil Science activities**

Mutation Breeding

Genotypes: 7/10
Locations: 3/5 (Peshawar, Kohat, DIKhan, KaraK, Chakwal)
Design: Randomized Complete Block
Replications: 4
Plot size: $9m^2$
Row Length: 5m
Row Distance: 30cm

Agronomic Traits:

Plant Height, Seed weight, Flowering, Seed Yield, Oil Yield

Biochemical Traits:

Oil, Protein, Glucosinolates, Erucic, Oleic, Linolenic Acids

Statistical Analysis:

Analysis of Variance (ANOVA) / Bi-Plot

Soil Science

- **Genotypes: Three (3)**
- **Experimental design: Split Plot**
- **Location: Two (Kohat/ Surezai Research Station) rain fed area**
    - **Replication:3**
    - **Plot size: $15m^2$**
- **Fertilizer:**

  N0: P0:K0 (kg ha^{-1})

  N50: P60:K60 (kg ha^{-1})

  N75: P60:K60 (kg ha^{-1})

  N100:P60:K60 (kg ha^{-1})

Experimental Detail

- **Phosphorus → Single Super Phosphate and K as (SOP) all at sowing time .**
- **Nitrogen →ordinary urea → will be applied in three splits at sowing, vegetative and flowering stages.**
- **N fertilizer utilization→^{15}N micro plots 1m^2 in each Treatment. Three micro plots will be created and 15 N labelled urea will be applied like one micro plot at sowing, vegetative and at flowering stages**
- **Labeled Urea required: 5% atom excess about 2 kg**
- **Sowing →hand drill**
- **Row-to-Row distance →recommended distance**
- **Weeds and insect pest control →recommended weedicides**

Parameters to be studied

- **Yield components and Crop Yield**
- **Seed and straw sampling at harvest from each crop for N and ^{15}N content**
- **N utilization from fertilizer N**
- **Water Use Efficiency**
- **Soil analyses for total organic C, N, available P,**
- **Meteorological observations**

  (Rainfall, Temperature, Humidity)

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