Case Study of Rice Biofortification through Mutation Breeding in Indonesia

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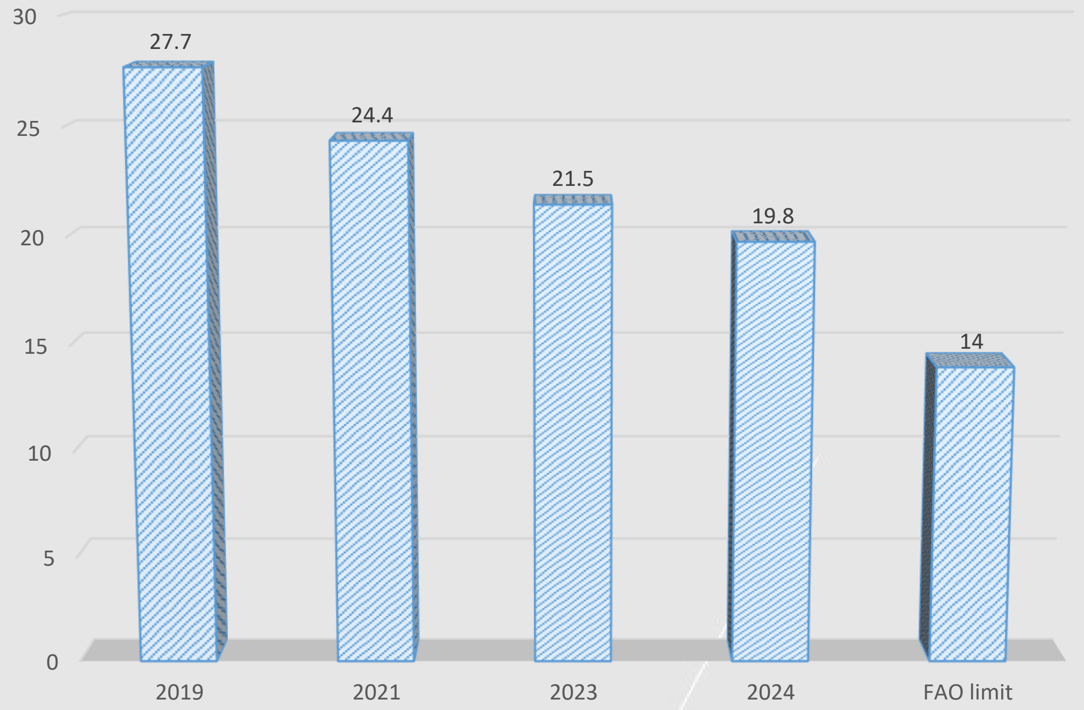
Regional Training Course dibawah kegiatan RAS5101 *Using Mutational Biofortification for Improving the Nutritional Quality of Food Crops*
IAEA

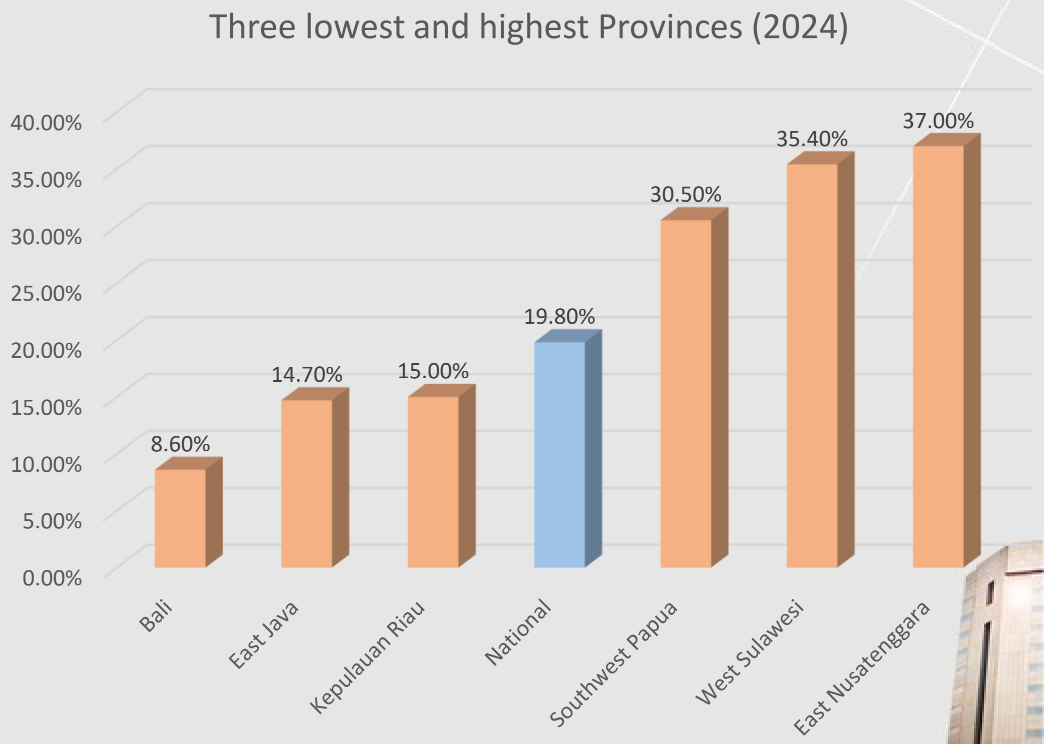
Bogor, 13 – 17th April 2026

BIOFORTIFICATION

- Hidden hunger in Indonesia: prevelence of stunting (18%, 2024), Fe (Anemia 16.2%, 2023), and Vitamin A (19.5%, 2022)
- Potential losses due to hidden hunger: decrease productivity, quality life, immunity, etc
- Anticipation: diverse diet, Supplementation, Fortification, **Bio**fortifikasi
- Biofortification: increasing of nutrition in the plant's edible part through **plant physiological mechanisms**

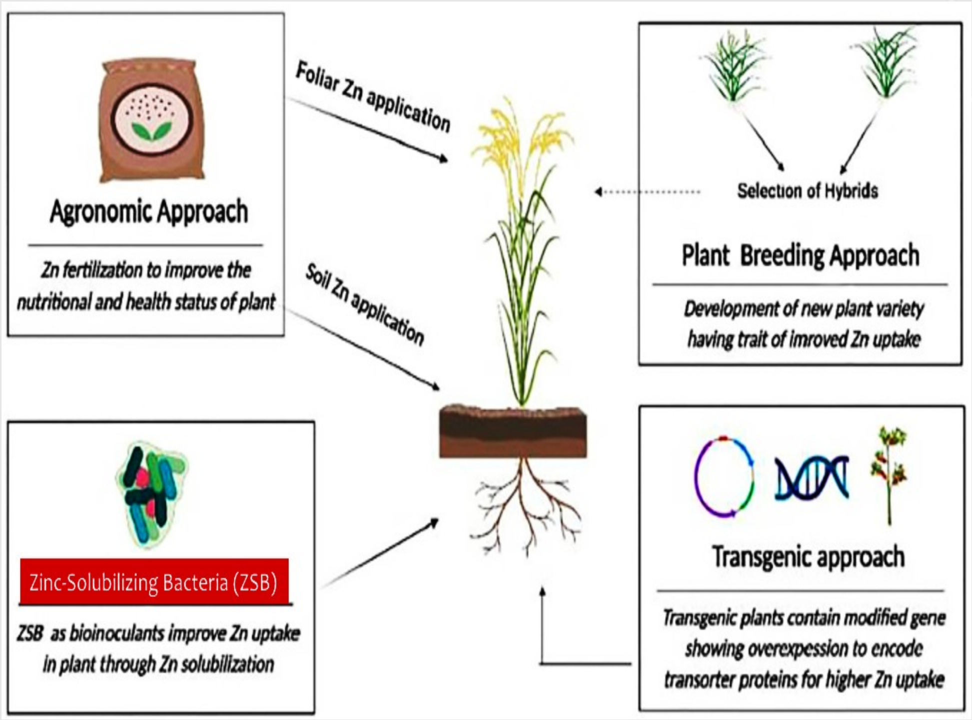
Prevalence of stunting in Indonesia





BIOFORTIFICATION APPROACHES

- Plant Breesing
  - Conventional: hybridization, chemical and/or physical mutation
  - Biotechnology: transgenic
- Agronomic:
  - Biological agens
  - Anorganic ferilizers



Rice for Indonesia

- **Staple food** for almost all Indonesian people (278 M people)
- Contribute to 40% calori and 30 protein intake
- Consumption/capita/year: 113 kg (2025)
- Rice field: 10.21 Mha
- Yield average: 5.6 t/ha
- Convertion rate: 180.000 ha/yr

Rice is a good vehicle to deliver nutrition to Indonesian people

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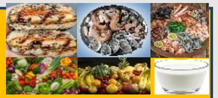
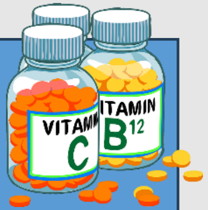
Why Biofortification Breeding?



- **Cost intensif**
- **Scaterred access**

DIET DIVERSIFICATION

SUPPLEMENTATION

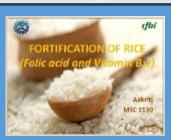


- Cost intensif
- Not sustaible
- Logistic delivery concern
- Diet cultural adaptation

FORTIFICATION

BIOFORTIFI CATION

(BREEDING)



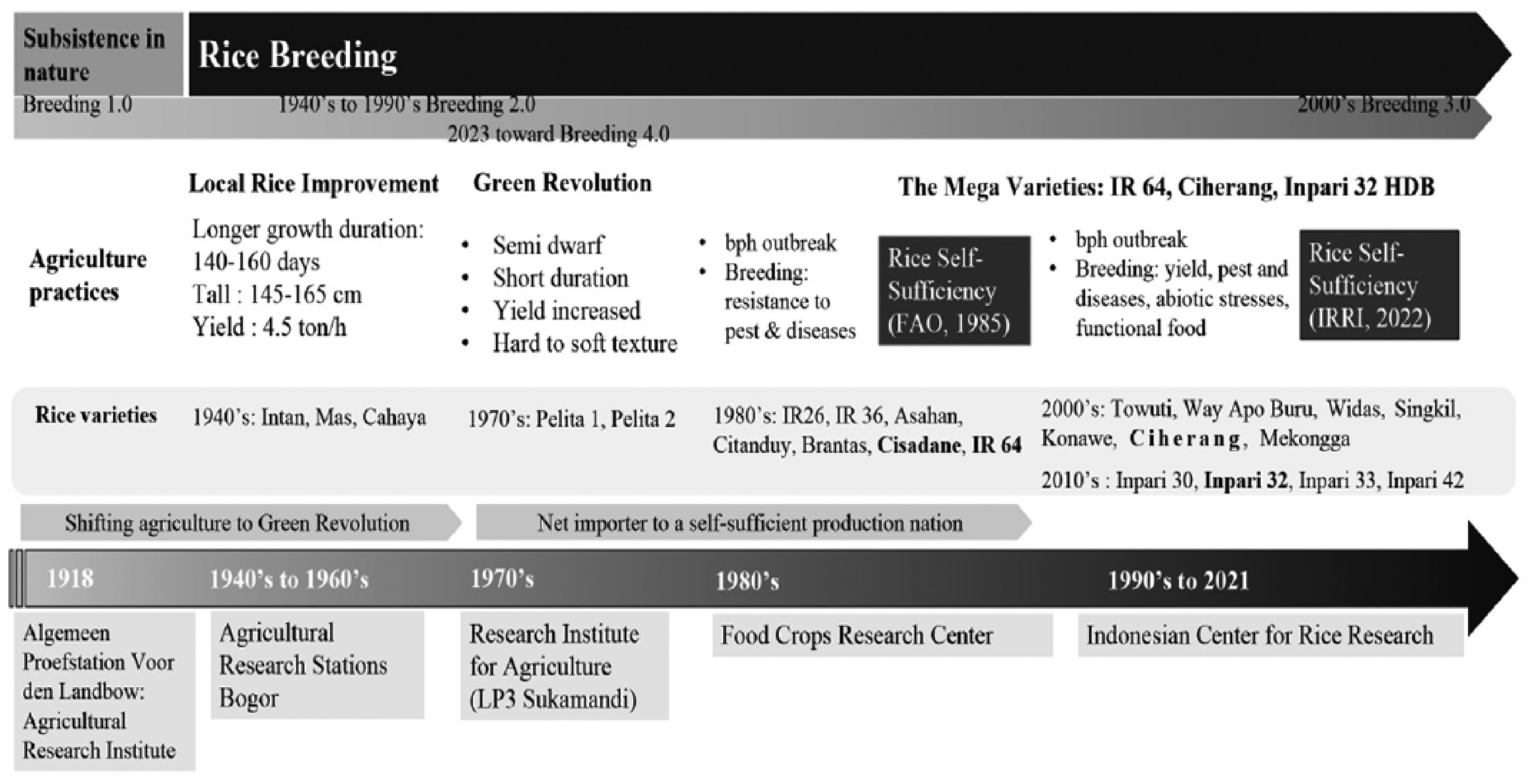
- Cost intensif
- Not sustaible
- Logistic delivery concern
- Diet cultural adaptation

- **Cost effective**
- **Sustaible**
- **Massive**
- **Natural**
- **Cultural adoption**

Rice Biofortification In Indonesia

- Agronomic biofortification
  - Foliar: $ZnSO_4$
  - Soil fertilizers: ZnO, $ZnSO_4$, some commercial fertilizers have Zn
- Genetic biofortification (Breeding)
  - Conventional
    - Hybridization: single cross, back cross
    - Mutation: gamma irradiation
    - Introduction: from IRRI
  - Biotechnology
    - Genome Editing: BRIN

Rice Biofortification Breeding



Rice varieties and research institutions involved in rice breeding history in Indonesia

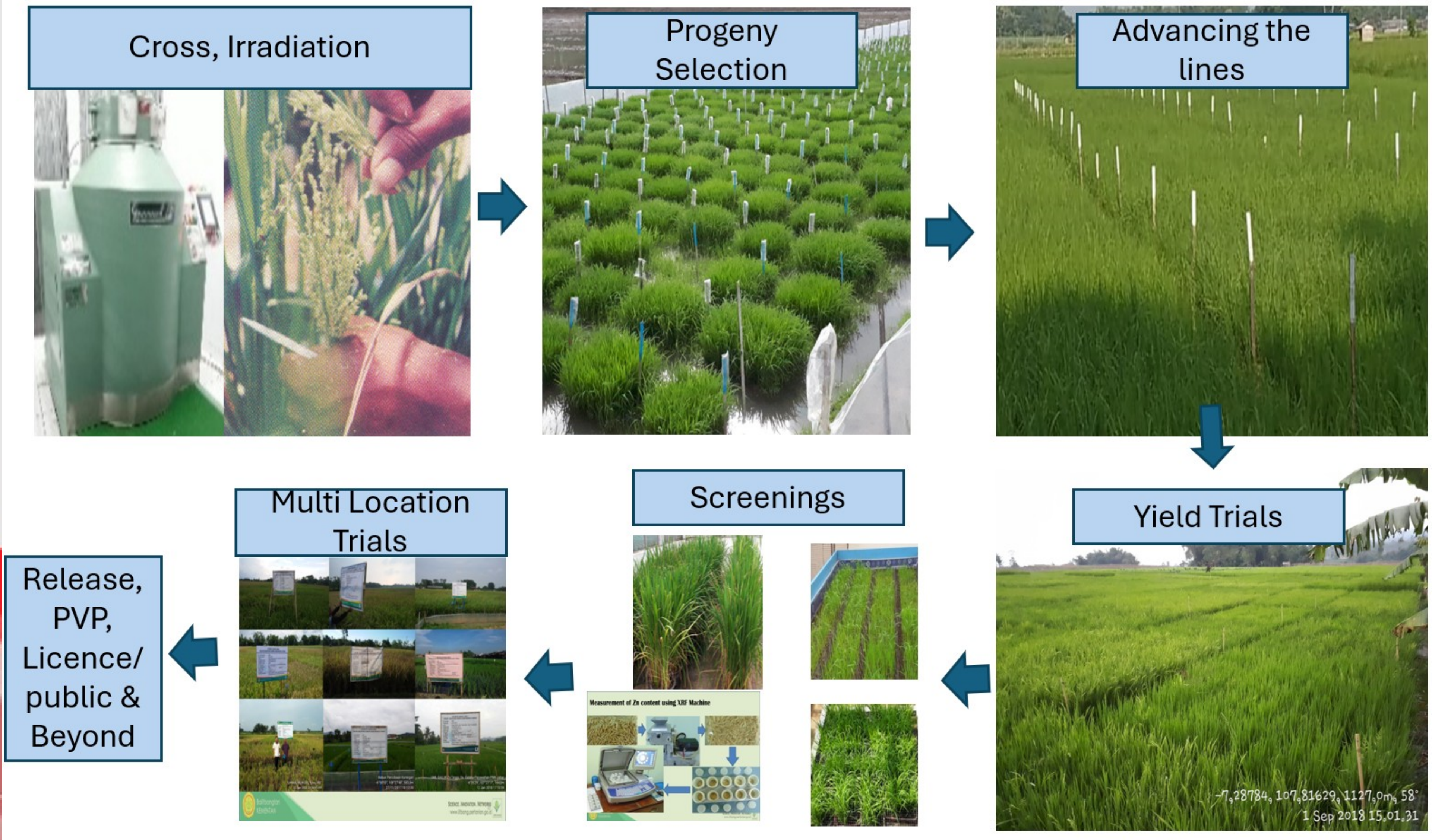
https://doi.org/10.1080/15427528.2025.2575385

Food and **Nutrition** Security

Inpari IR Nutri Zinc
Inpago 13 Fortiz
Inpara 11 Siam HiZinc
Inpara 12 Mayas
…

BRIN
Universities
…

Rice Breeding Flow in Common



Mutation Rice Breeding in Indonesia

- National Nuclear Energy Agency of Indonesia (**BATAN**) lead the mutational crop breeding efforts.
  - Some of the varieties: Atomita 1 (1982), Atomita 2 (1983), Atomita 3 (1990), Atomita 4 (1991), Cilosari (1996), Meraoke (2001), Woyla (2001), Kahayan (2003), Winongo (2003), Diah Suci (2003), Yuwono (2004), Mayang (2004), Mira 1 (2006), Bestari (2008), Pandanputri (2010), Inpari Sidenuk (2011), Inpari Mugibat (2012), Suluttan Unsrat 1 (2012), Suluttan Unsrat 2 (2012), Situgintung (upland, 1992), Sinar 1 (2020), Sinar 2 (2020), Lampai Sirendah (2020), Isora (2021), Dayang Muratan (2021), Dayang Muratan 4 (2021), Payo Ngarayak (2021), Payo Ilik Aso (2021), Pikatan (2023), …
  - No biofortified released variety as the trade mark
- Indonesian Agency for Agricultural Research and Development released some gamma irradiate mutant rice varieties:
  - Munawacita Agritan (2017), Mustaban Agritan (2017), Cakrabuana Agritan (2018), **Inpara 12 Mayas (2020; Hi Zinc)**

GAMMA IRRADIATION MUTATION FOR BIOFORTIFICATION

- Some breeding materials (2026):

Plot	Source	Origin
	M4	
M-401	M-301	Karang Dukuh M200
M-402	M-302	Margasari M200
M-403	M-303	Margasari M300
M-404	M-304	Logawa M200
M-405	M-305	76 (Mira Mutant)
	M5	
M-501	**M-401**	**Inpari IR Nutri Zinc 200Gy**
M-502	**M-402**	**Inpari IR Nutri Zinc 300Gy**
M-503	M-403	Inpari 48 Blas 200Gy
M-504	M-404	Inpari 48 Blas 300Gy
	M1	Lapang Pangkep
M-101		Lapang 200 Gy
M-102		Lapang 300 Gy

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Biofortified Inpari 32 HDB Mutans

- Generation M7
- Best mutant vs original variety:
  - 1000 grain weight: 28.21 g vs 26.40 g
  - Fe 52.26 ppm vs 26.71 ppm
  - Zn 53.89 ppm vs 27.01 ppm
- Ready for further yield trials

GENOME EDITING

- Gene of target: *osVMT*
- Vehicle: Crispr Cas9
- Wild variety: Kitaake (Japonica)
- Progress: T1 to T2 mutant lines; confirming of phenotype and gene expression
- Challenges:
  - Generation ability of Indica genetic background
  - Stability of mutant over generations

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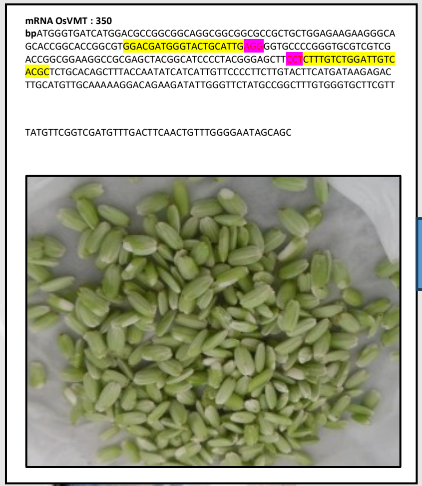
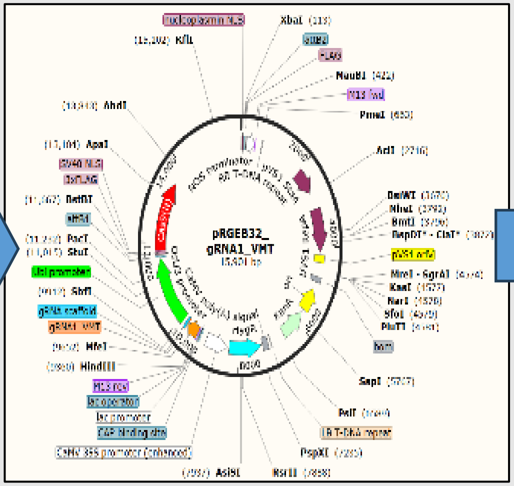
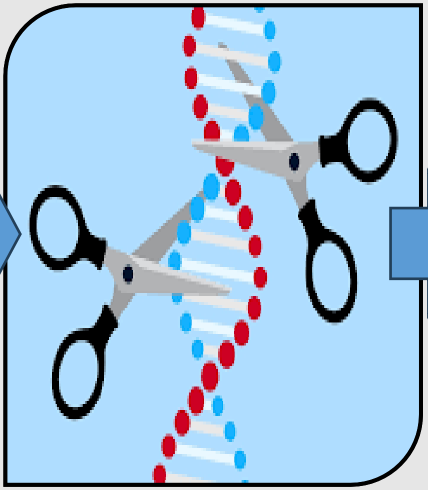
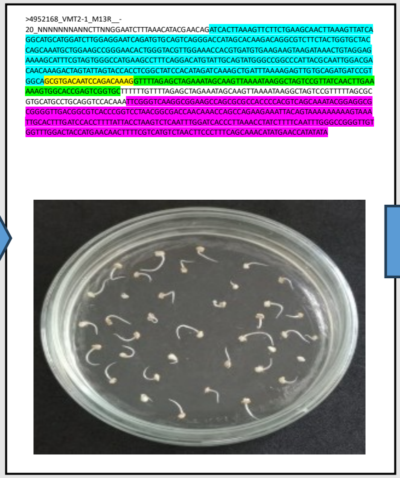
GENOME EDITING

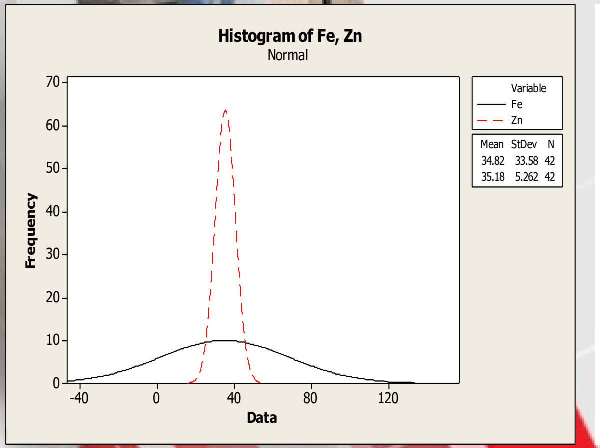


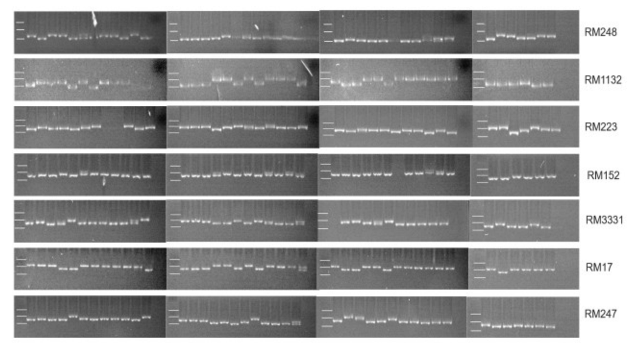


Target: *OsVmt*









Lampiran Gambar 2. Hasil elektroforesis dari RM248, RM1132, RM223, RM152, RM3331, RM17, RM247

Varietas	Galur
Kitaake	63
Nipponbare	12
Mekongga	Low regeneration
Inpari 32	Low regeneration

GAMMA IRRADIATED MUTANT BIOFORTIFIED RICE VARIETY

Inpara 12 Mayas (3516/HK.540/C/12/2022)

Growth Duration : 117 days after sowing
Plant growth : 112-115 cm
Grain shape : Slender
Texture : Medium soft
Yield average : 4.88 t/ha
Yield potential : 8.44 t/ha
Tolerance : Medium tolerant to Fe toxicity, Medium resistant to blast
Zn content : 29 ppm (brown rice)
Cultivation : ICM

Swampy

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OTHER RELEASED BIOFORTIFIED RICE VARIETIES

Inpago 13 Fortiz

(SK No. 990/11 November 2020)

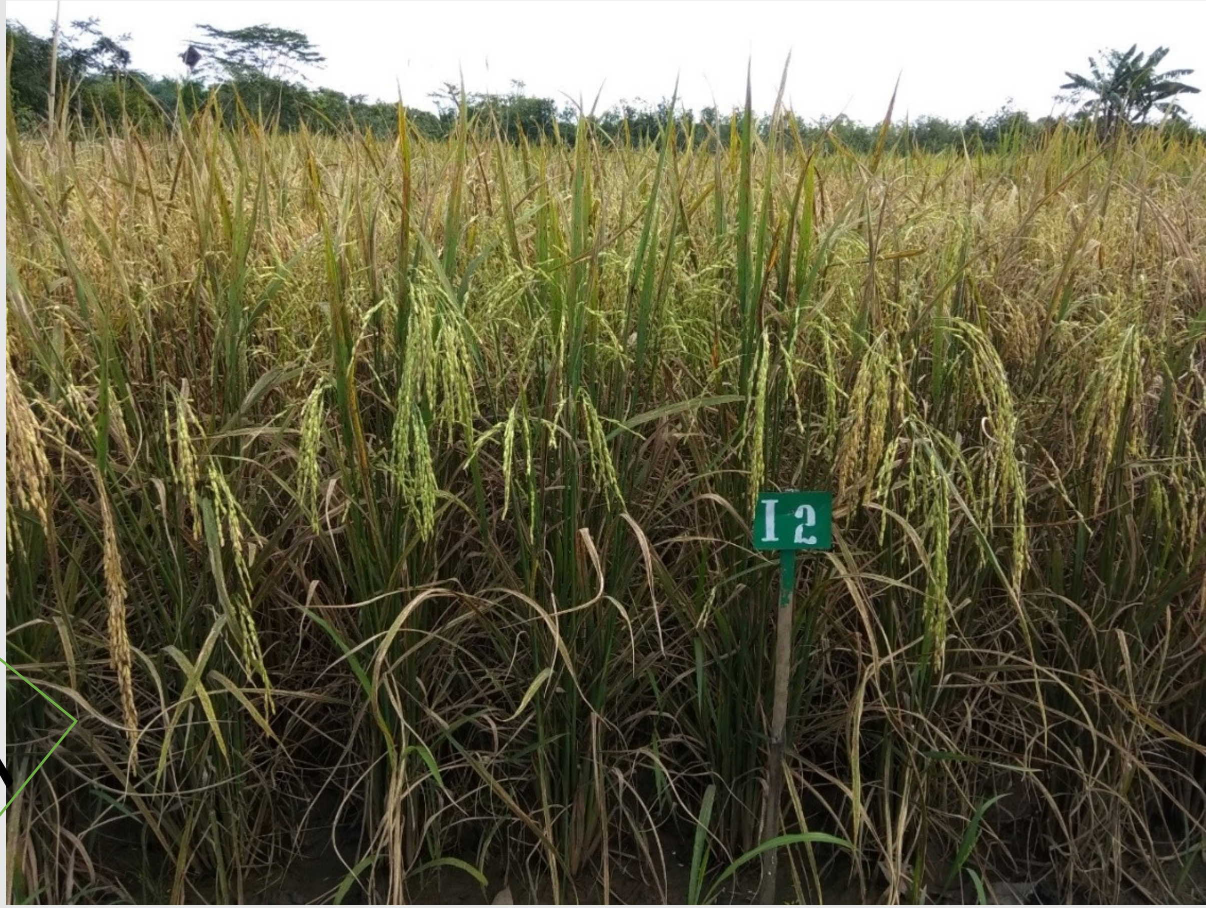
- Yield average : 6.53 t/ha
- Yield potential: 8.11 t/ha
- Protein content: 9.83 %
- Potential of Zn content : 34 ppm
- Other traits:
  - Medium resistant to 8 blast isolates
  - Medium resistant to Al toxicity
  - Medium tolerant to drought stress
  - Cultivation : ICM

Upland

Inpara 11 SIAM Hizinc

(168/HK.540/C/01/2019)

- Growth Duration : 122 days after sowing
- Plant height : 115 - 120 cm
- Grain shape : Slender
- Texture : Medium soft
- Yield average : 4.83 t/ha
- Yield potential : 6,07 t/ha
- Resistance : Medium resistant to BB and Blast
- **Zn content (>30 ppm)**
- Cultivation : ICM



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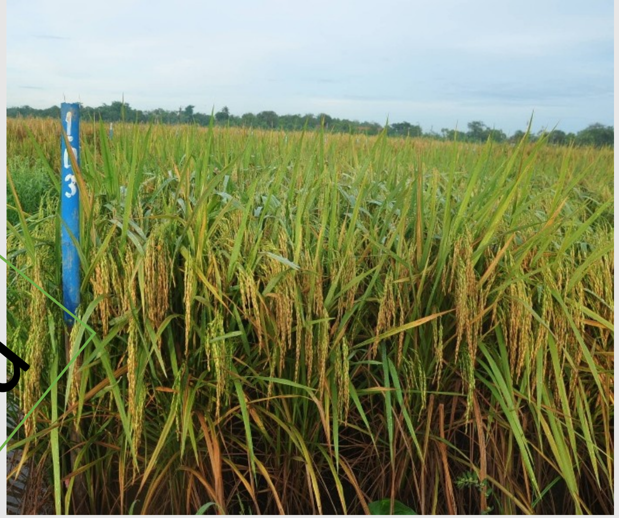
Inpari IR Nutri Zinc

(168/HK.540/C/01/2019)

- Yield potential: 9.98 t/ha
- Productive tiller: 18 tiller
- Textur: medium soft
- 1000 grain weight: 24.60 g
- Average Zn content: 29.54 ppm
- Potential Zn content: 34,51 ppm
- Resistant: MR BPH 1 and 2, MR BB III, R Blas 033, 073, 133, MR Tungro Garut dan Purwakarta
- Cultivation : ICM

Irrigated



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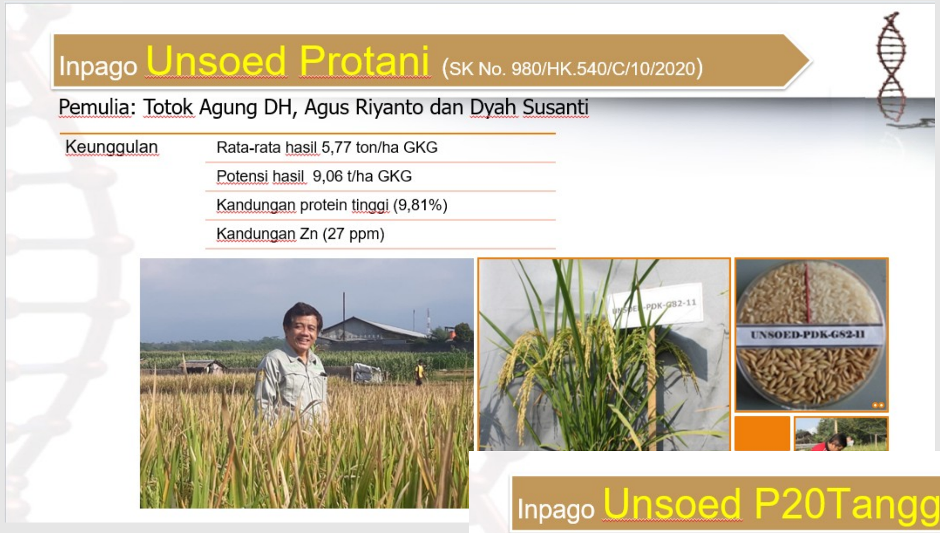
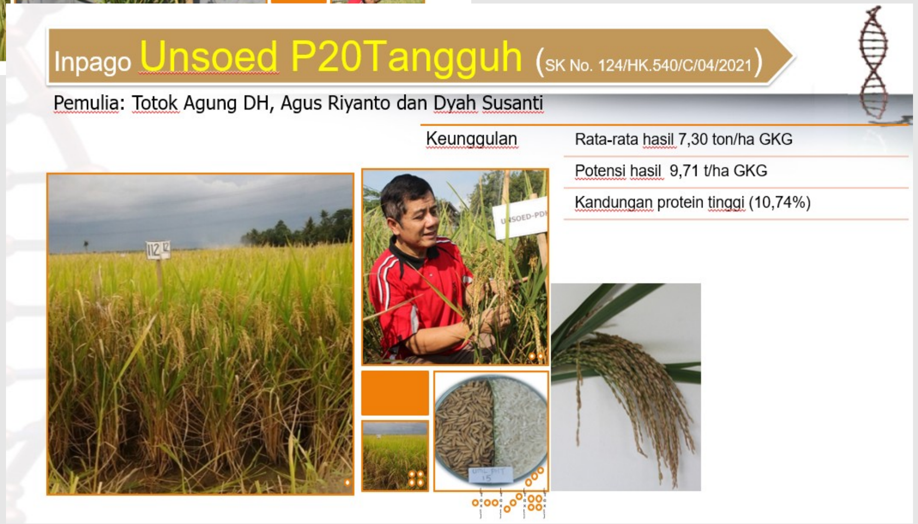
High Protein Rice

- Inpago Protani (9.81 %)
- Inpago 13 Fortiz (9.83 %)
- Unsoed P20 Tangguh (10.4 %)





High Fe Rice

- Inpari 5 Merawu (33 ppm)
- Unsoed Parimas (27 ppm)

High Anthocianine Rice

- Inpari 24 Gabusan
- Inpari 25 Opak Jaya
- Jeliteng
- Arumba
- Pamera
- Pamelen

Beras merah Inpari Arumba. Sumber: bbpadi.litbang.pertanian.go.id.

Some Lessons Learned

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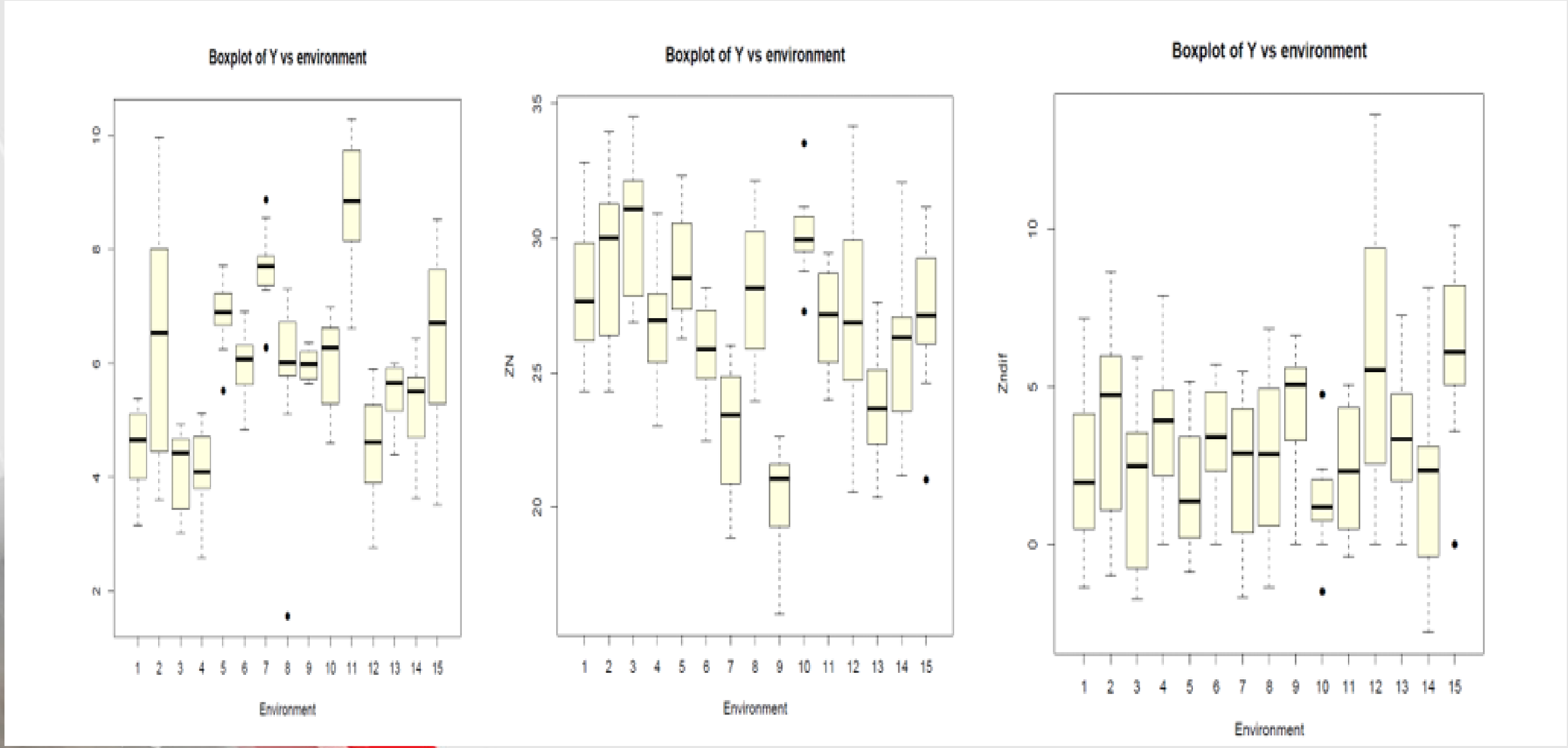
GxE for grain Zn content



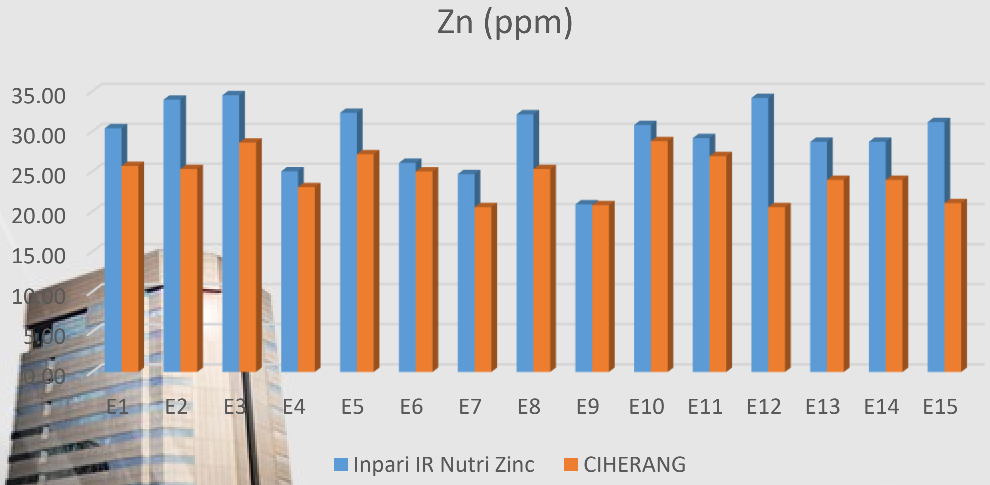
Yield (t/ha)

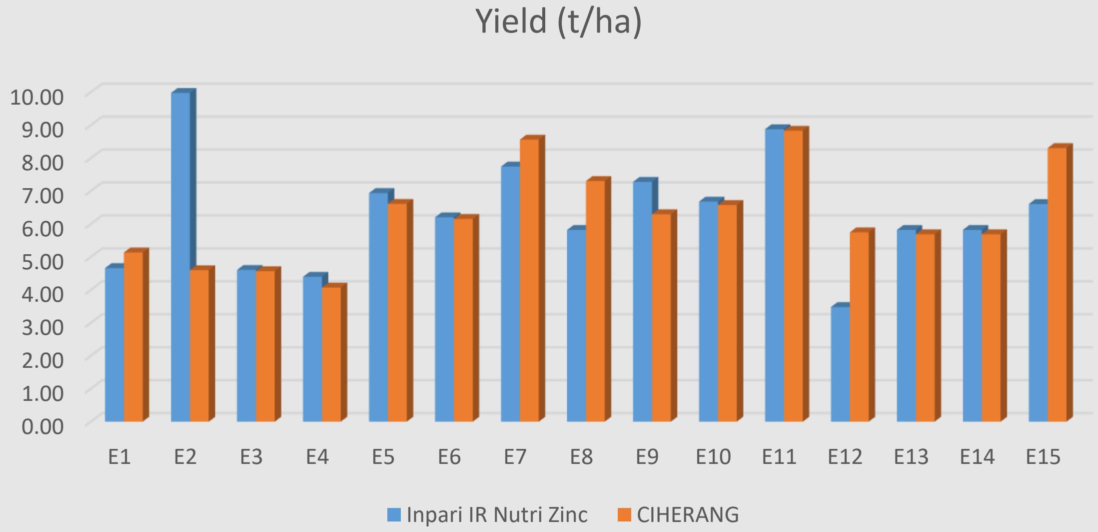
Zn (ppm)

Zn deviation from Ciherang (ppm)

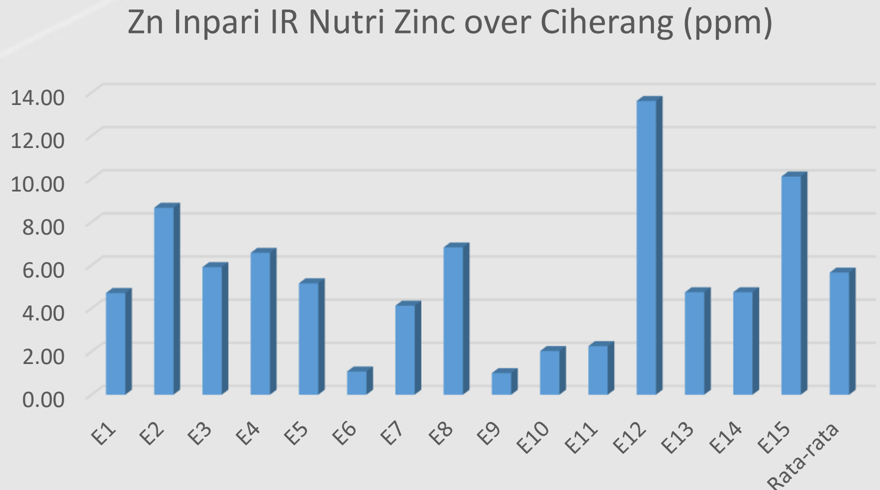
E1=Indramayu WS 2016;
E2=Karawang WS 2016;
E3=Subang WS 2016;
E4=Sukoharjo WS 2016;
E5=Sumedang WS 2017;
E6=Deli Serdang WS 2017;
E7=Serdang Bedagai WS 2017;
E8=Purwakarta WS 2017;
E9=Kediri WS 2017;
E10=Nusa Tenggara Barat WS 2017;
E11=Karawang DS 2017;
E12=Kuningan DS 2017;
E13=Cilacap DS 2017;
E14=Nusa Tenggara Barat DS 2017;
E15=Malang DS 2017.

Zn content increase
Inpari IR Nutri Zinc vs Ciherang





source: MLT 2016 – 2017



Variation among environment is existed

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Phytic Acid Contradictive

Rohaeni *et al.* (2023)

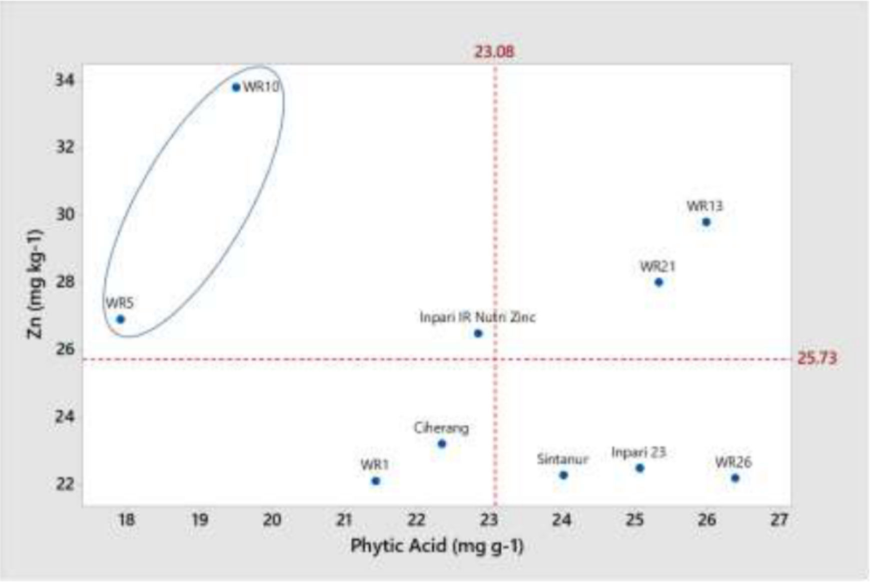

Figure 1. Scatter plot of relationship between phytic acid and Zn of rice lines and cultivars. Red lines X, Y = mean value of phytic acid (mg g^{-1}) and Zn (mg kg^{-1}), respectively.

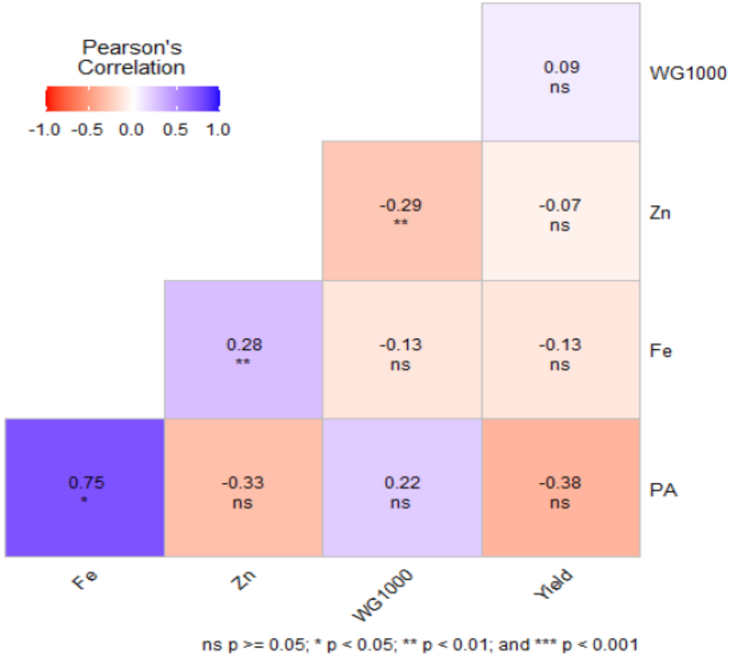

Figure 3. Pearson's correlation coefficients between phytic acid (PA) characters with Fe, Zn, 1000-grain weight (WG1000), and grain yield per plant in rice lines and cultivars. Significance of correlations indicated as ** $P < 0.001$; *$P < 0.01$; ns, not significant.

- PA bind metal ions makes not available for human body (reduce Fe and Zn bioavailability)
- Hig Fe/Zn low PA is ideal

RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
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http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

SEAMEO SEARCA

PHYTIC ACID CONTENT IN BIOFORTIFIED RICE LINES AND ITS ASSOCIATION WITH MICRONUTRIENT CONTENT AND GRAIN YIELD OF RICE

W.R. ROHAENI[1,2*], TRIKOESOEMANINGTYAS[4], U. SUSANTO[3], M. GHULAMAHDI[4], W.B. SUWARNO[4], and H. ASWIDINNOOR[4]

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Post Harvest Study

- Milling reduces Zn by 20%

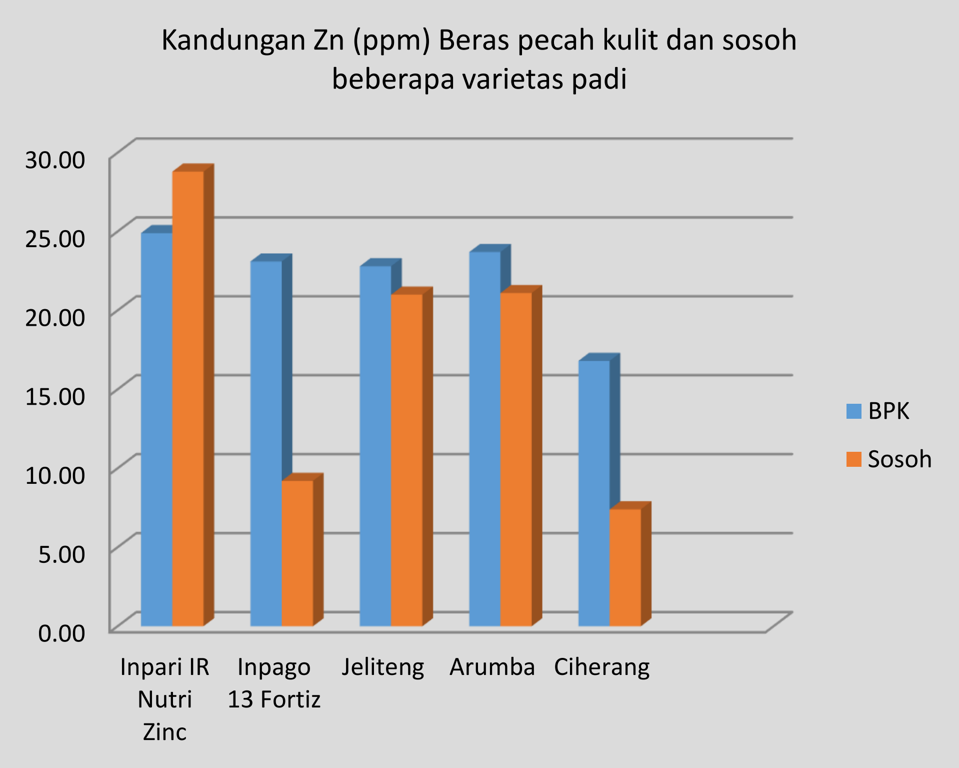
No.	Sampel	Brown Rice*	Polished Rice*
1	Inpari IR Nutrizinc Non Pratanak	35,5±0.1^{a}	29,4±0.1^{b}
2	Inpari IR Nutrizinc Pratanak	36,9±0.4^{a}	28,6±0.2^{bc}
3	Ciherang Non Pratanak	27,7±0.9^{bc}	22,0±0.6^{d}
4	Ciherang Pratanak	26,8±0.4^{c}	21,4±0.5^{d}

- Penggunaan kukusan kandungan Zn cenderung lebih tinggi daripad rice cooker (perbedaan sangat tipis)

No.	Sampel	Nasi BPK Kukusan	Nasi BPK Rice Cooker	Nasi Sosoh Kukusan	Nasi Sosoh Rice Cooker
1	Inpari IR Nutrizinc Non Pratanak	16.3±0.5^{a}	16.1±0.6^{a}	10.4±0.0^{def}	10.1±0.3^{defg}
2	Inpari IR Nutrizinc Pratanak	17.4±0.4^{a}	16.1±0.1^{a}	10.6±0.1^{de}	10.2±0.1^{defg}
3	Ciherang Non Pratanak	14.6±0.3^{b}	13.5±0.1^{bc}	9.4±0.1^{efg}	9.2±0.6^{fg}
4	Ciherang Pratanak	13.0±0.4^{c}	11.5±0.5^{d}	9.0±0.1^{g}	8.8±0.4^{g}

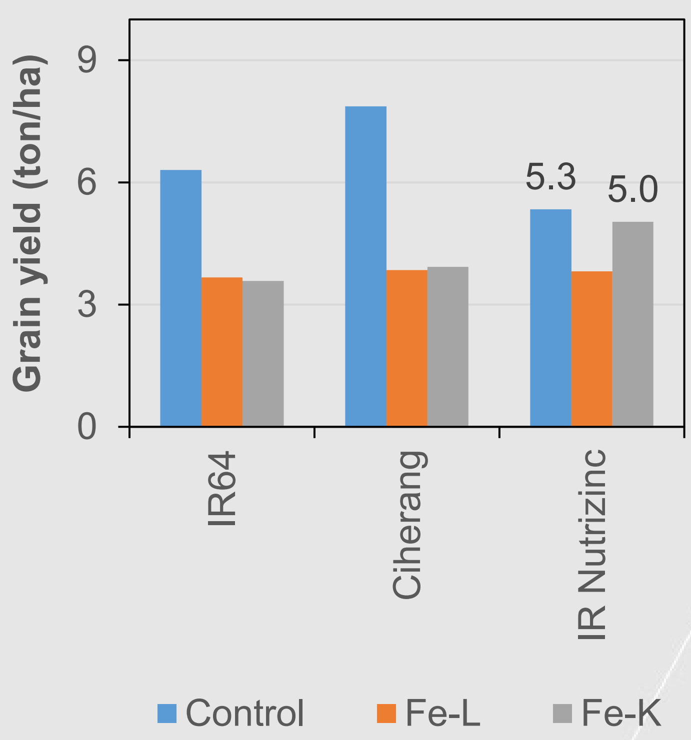
- Penurunan kandungan Zn karena penanakan:
  - Beras pecah kulit: 53% (tersisa 47%)
  - Beras sosoh: 61% (tersisa 39%)

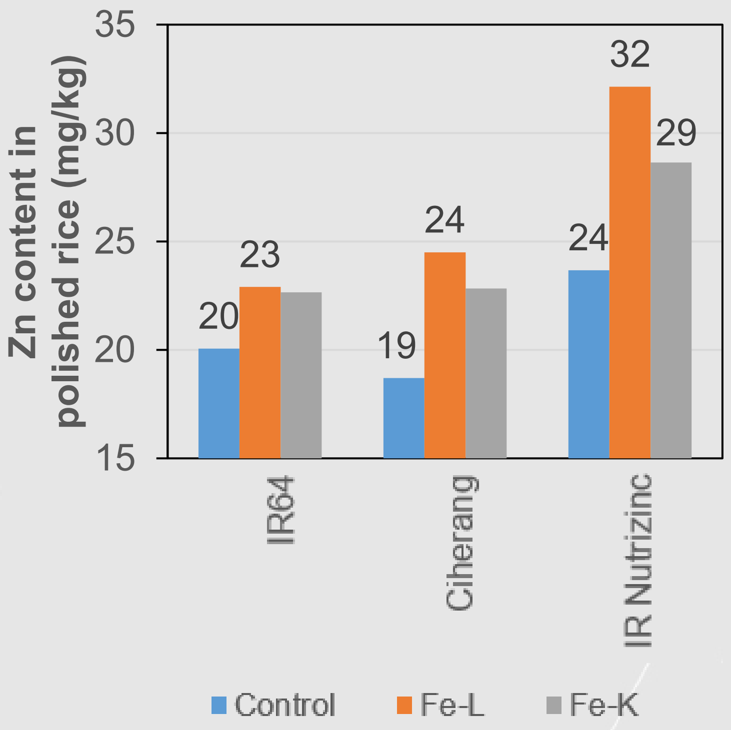
- Penurunan kandungan Zn karena proses panca panen terjadi pada semua varietas



Courtacy Kementrian Pertanian

Grain Zn content and soil Fe interaction





Inpari IR Nutri Zinc

→ Superior yield under Fe toxicity

→ Superior Zn content in both optimum and Fe toxic condition

Rosdianti et al., in press

Inpari IR Nutri Zinc is prospective for Fe toxic area

EFFICACY STUDY OF FE BIOFORTIFIED LINES

Tabel 2. Asupan Zat Gizi Harian, Status Gizi, Status Besi, dan Kebugaran

Hasil	Sebelum Intervensi[1]	Setelah Intervensi[1]	*p*
Asupan gizi/hari			
Energi (kkal)	2.156,6 ± 764,4	1.579,7 ± 374,5	0,001*
Karbohidrat (g)	260,4 ± 88,7	189,2 ± 50,9	0,001*
Protein (g)	57,4 ± 21,4	45,6 ± 12,2	0,017*
Lemak (g)	96,4 ± 40,7	70,1 ± 17,1	0,008*
Besi (mg)	6,7 ± 2,9	9,7 ± 1,9	0,000*
Kalsium (mg)	271,1 (140, 422)	82,2 (61, 103)	0,001*
Asam folat (µg)	163,9 ± 86,1	78,1 ± 45,9	0,001*
Vitamin B12 (µg)	2,7 (1, 3)	2,7 (2, 3)	0,420
Vitamin A (µg)	325,5 ± 168,1	203,8 ± 144,4	0,008*
Vitamin C (mg)	5,0 (3, 10)	5,4 (2, 8)	0,528
IMT/U (skor Z)	0,6 ± 0,8	0,6 ± 0,7	0,058
Kadar hemoglobin (g/dL)	12,3 ± 1,1	11,9 ± 1,2	0,012*
Kadar ferritin serum (µg/L)	9,9 (7, 22)	10,9 (7, 31)	0,094
VO_2max (ml/kg/menit)	36,1 (35, 41)	36,4 (34, 39)	0,018*

[1]Nilai disajikan dalam bentuk rata-rata ± standard deviasi atau median (jangkauan interkuartil). *Berbeda signifikan (p< 0,05) dengan Paired t-test atau Wilcoxon Signed Ranks Test.

Rachmah et al. Media Gizi Indonesia (National Nutrition Journal). 2022.17(3): 320–329 320
https://doi.org/10.204736/mgi.v17i3.320–329

PENGARUH INTERVENSI BERAS TINGGI BESI TERHADAP VO_2MAX SANTRIWATI PONDOK PESANTREN AL-FALAK BOGOR

Effect of High-iron Rice Intervention on VO_2max of Female Students of Al-Falak Bogor Islamic Boarding School

Ade Salma Yunia Rachmah [1], Mira Dewi[1*], Evy Damayanthi[1], Dwinita Wikan Utami[2]
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*E-mail: mirade@apps.ipb.ac.id

- No Fe blood increase, but better in physical fitness

Fe Bioavailability Study

TABLE 2. The iron content of some rice lines was observed in this study.

No.	Number	Genotype background	Total Fe (Fe^{3+} & Fe^{2+}) (ppm)*	Fe Soluble (Fe^{3+}) (ppm)	% bioaccessibility
1.	F47-C	Pulut siding-19	40.2	13.4	33.32
2.	5R-B	B11844-MR-29-7-1 / Ina5 // Code	30.1	12.8	42.52
3.	5R-C	B11844-MR-29-7-1 / Ina5 // Code	31.9	11.8	36.98
4.	10R-B	Ina4/Mekongga	41.2	20.6	49.95
5.	10R-C	Ina4/Mekongga	62.2	11.6	18.65
6.	1R-C	KDM-105-9 / B13143-8-MR-3-KA-14 // Ina5	50.4	16.6	32.92
7.	14R-C	Ini9 / Ina4	32.2	16.2	50.25

Note: *) Fe content on polished grain; **) The Fe content on the two varieties controlled was noted based on previous research, on Mahsuri (Anuradha et al., 2012) and IR64 (Gregorio et al., 2000). The lines ended with annotation B or C indicating the sample collected from location B (Belitang) or location C (Citayam); Ina4 and Ina5 were released as swampy rice varieties and as a donor parents for iron characters; Code, Mekongga, and Ini9 are released as elite rice varieties; BL: breeding lines, IL: Introduction lines.

The lines have good Fe bioaccecability

2024

Molecular Breeding of Biofortified Rice and Its Bioaccessibility on Cooked Rice

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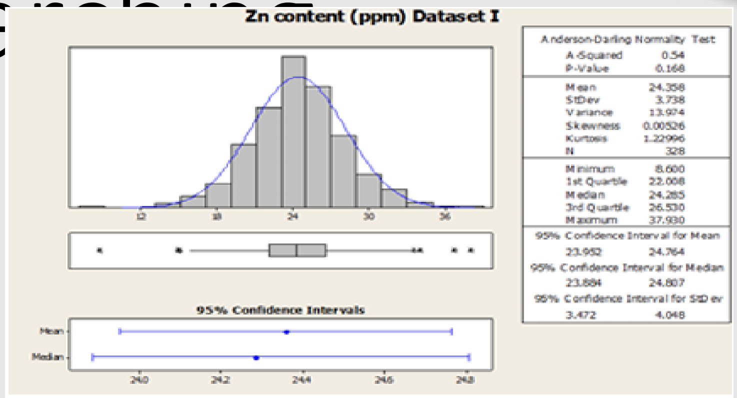
BRIN
BADAN RISET
DAN INOVASI NASIONAL

PROFESIONAL
OPTIMIS
PRODUKTIF

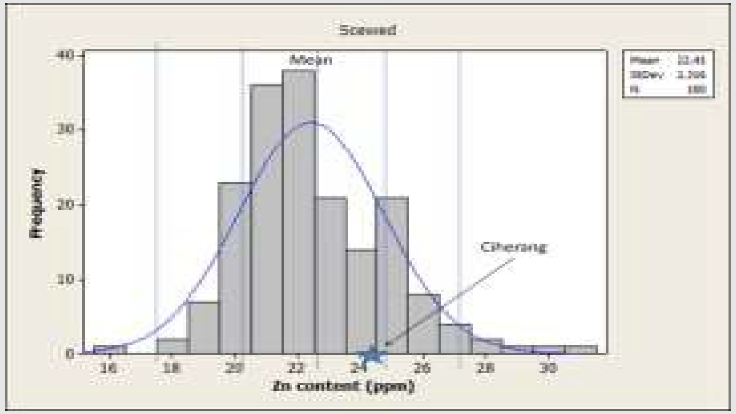
Everlasting new donor sea

- Germ plasm collection: 328 accessions, for Zn (Widyastuti *et al*, 2024):
  - 8.6 ppm (Ijo Gading) - 37.93 ppm (Pare Ndele), average 24.36 ppm (EDXRF)
  - 6.60 ppm (Mira 1) - 21.10 ppm (Biobestari), average 11.86 ppm (TXRF)
- Improved genotypes: 176 vareities + 4 lines (Rohaeni and Susanto, 2021):
  - 15.80 (Inpago 7) –31.10 ppm (Kalimutu), average 22.41 ppm; *Ciherang* (24.70 ppm, menengah)



Searching of new gene donors for high Fe and Zn content from Indonesian rice varieties

Y Widyastuti[1], M Santoso[2], H Aswidinnoor[3], Suprayogi[4], E Oktaviani[4], I S Dewi[1], Sobrizal[5], K Y Nasution[5], J Prasetyono[6], S D Indrasari[7], G R Pratiwi[1], E Apriyati[7], Mahrup[6], N Yunani[1], C Suparman[1], I M J Mejaya[1], P H Sinaga[1], Aryanti[5], T R D Larasati[5], J Mellawati[8], Tasliah[6], Efendi[9], R H Wening[10], W R Rohaeni[10], B P M Swamy[11], U Susanto[1*]



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Fe and Zn content of various genetic background of released rice varieties in Indonesia

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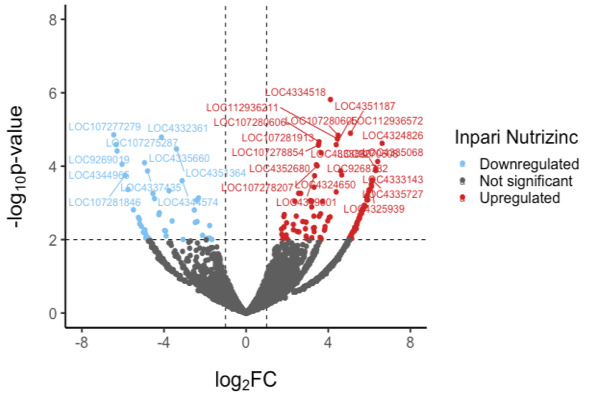
Trancriptomic profiling Inpari IR Nutri Zinc vs Inpari 32 HDB

PROFESIONAL OPTIMIS PRODUKTIF

Differentially express genes (DEGs) of Inpari 32 vs Inpari Nutrizinc from seed sample



DEGs of Inpari32 HDB vs InpariIR Nutri Zinc from four different samples

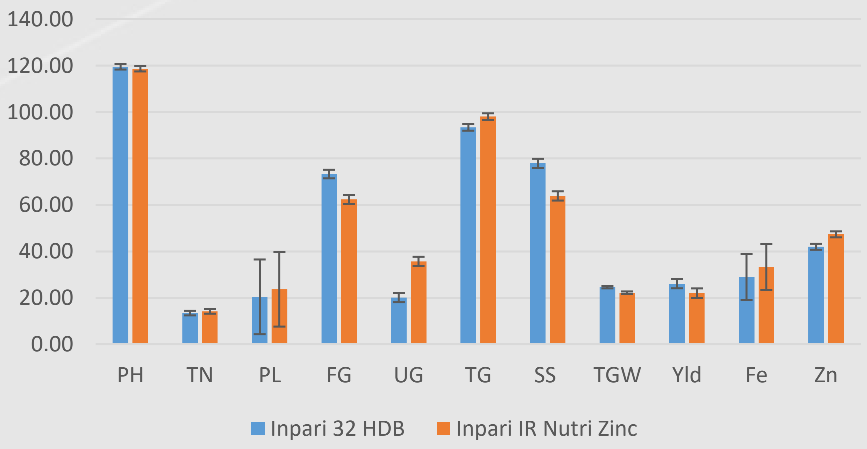
Samples	Up-Regulated	Down-Regulated
Seed	130	47
Leaf	439	157
Stem	561	169
Root	385	150

Log2 fold change: 1, p-value: 0.01

List of gene associated with zinc and/or metal ion binding among the list of up-regulated DEGS of Inpari IR Nutri Zinc vs Inpari 32 HDB from seed, leaf, shoot, and root samples

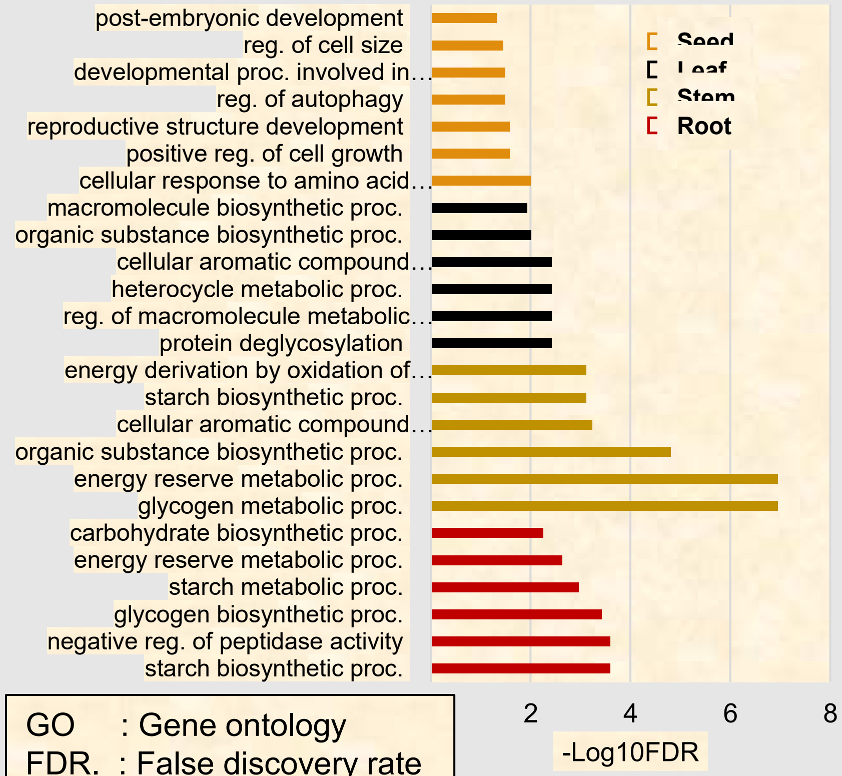
Entrezgene	Gene.Symbol	Rapdb	Description
4349156	LOC4349156	Os10g0523100	2-oxoglutarate-Fe(II) type oxidoreductase hxnY
4329193	LOC4329193	Os02g0327100	ADP-ribosylation factor-like protein 2
4325939	LOC4325939	Os01g0713600	B3 domain-containing protein LFL1-like
4349698	LOC4349698	Os11g0134300	CBL-interacting protein kinase 33-like
4325028	LOC4325028	Os01g0898900	CMP-sialic acid transporter 3-like
4344236	LOC4344236	Os07g0669800	uncharacterized LOC4344236
4348450	LOC4348450	Os10g0363300	acetyl-CoA carboxylase 1-like
4349064	LOC4349064	Os10g0506100	heavy metal-associated isoprenylated plant protein 39
107276004	LOC107276004	Os02g0599150	probable protein phosphatase 2C 16
4333130	LOC4333130	Os03g0421000	uncharacterized LOC4333130
4350304	LOC4350304	Os11g0292050	uncharacterized LOC4350304
....	....	....	....

Phenotypic differences between Inpari IR Nutri Zinc and Inpari 32 HDB



Up-Regulated genes

GO Biological Processes



GO : Gene ontology
FDR. : False discovery rate

Genes associated with Zinc (Zn) or Iron (Fe) transport in InpariIR Nutri Zinc;

- *OsZIP9* (Zn)
- *OsNRAMP5* (Fe)

CHALLENGES

- Variety
  - Limited amount and genetic variation for the diverse requirements (environment, people preference, etc)
  - Nature of Genetic x Environment interaction; different mindset with industrial business world
    - No fixed value to consider high or low; depend on location and check variety
    - Deviation from check also vary among locations
- Adoption
  - No single trait superiority is enough for adoption
  - Yield: compared to the most popular high yielding variety in the location
  - Quality > palatability: amylose content (soft vs hard adoption locations)
- Socio-economic
  - Awareness of people, entrepreneur, government
  - Political will (variation among regions)
- Climate Change:
  - Increase temperature increase grain Zn content, but reduce yield
- Phenotyping (measuring Fe/Zn content):
  - Expensive
  - High contamination risk
  - Need specialized equipment (reliability among labs)

PROSPECT

- Advantageous: sustainable, massive, economic, practical, *effective*
- Increasing awareness of health concern
- Had become and hopefully continuous as governmental program and priorities
- National priority program to combat stunting
- National program of MBG
- Continuous breeding efforts having prospective materials in the pipe line

BRIN

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TERIMA KASIH