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Gene Effect Estimation in *Brassica juncea* through Generation Mean Analysis

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ABSTRACT
India's major oilseed crop is Brassica juncea. It makes up more than 80% of the nation's total production of rapeseed-mustard and 27% of its edible oil reserve. A plant breeder would find information on gene effects very helpful in selecting the best breeding strategy for quicker advancements. In Brassica juncea generation mean analysis, two crosses, PWR 15-8×RGN73 (Family A) and PWR 15-8×PM25 (Family B), were used to investigate the type and extent of gene effects for yield and its components. The P1, P2, F1, F2, BC1, and BC2 generations were examined for fifteen quantitative characteristics linked to yield and oil quality. All the parameters under investigation demonstrated significant variability within families (among progenies), but only the plant height, days to flower initiation, oil content, glucosinolate content and protein content, showed significant variability between families. An adequacy test of multiple models for traits revealed that an epistatic model was adequate for every attribute in both families. The estimates of gene effects under a suitable model showed that genes effects were important in the two families for protein content, days to maturity, and secondary branches/ plant, in addition to one or more epistatic effects, such as dominance [h] and additive [d]. These factors would only be taken into account for the following: in Family-A, only for plant height, length of main raceme, siliqua density, , glucosinolate content and oil content; in Family-B, only for days to flowering and seed yield/ plant. It was suggested that selection should be postponed for larger gains until later generations due to the existence of fixable effects, namely additive and additive-additive effects, for plant height, number of secondary branches/plant, siliqua length, and 1000-seed weight in Family-A and days to maturity, oil content, protein content, and glucosinolate content in Family-B, respectively.
Key words: *Gene effect, Brassica juncea, quantitative traits*

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INTRODUCTION

The Brassicas commonly known as Rapeseed-Mustard are important group of edible oils and vegetables crops belonging to Brassicaceae family. This group comprises of six cultivated species, namely, *Brassica rapa*(2n=20, AA), *Brassica nigra* (2n=16, BB) and *Brassica oleracea (*2n=18, CC*)* with diploids genome; *Brassica juncea*(2n=36, AABB), *Brassica napus (*2n=38, AACC*) and Brassica carinata(*2n=34, BBCC*)*with tetraploids genome size. The hybridization of the component diploid species and subsequent chromosomal doubling led to the evolution of amphidiploids in nature. Brassica juncea is one of these species and an important oilseed crop in India. It provides more than 80% of the nation's rapeseed-mustard output as well as 27% of its edible oil supply [1].

A breeding programme always aims at the increment in seed yield to cater the demand of increasing populations. Yield is the ultimate product of action and interaction of number of yield components traits which are govern by a large number of genes having small effects and are greatly influenced by environment. The gain in seed yield can be achieved by adopting suitable breeding methods which allow most desirable combinations of genes for maximum possible production. Breeding methods are determined by the gene action, interaction and linkage relationship of genes which conditioned the phenotypic variation of various metric traits. Thus, both the components of genetic variance i.e. additive and non-additive, along with their allied parameters are of immense use for a plant breeder under

different situations. Both the components of genetic variance help in determining a suitable selection procedure and more fruitful breeding programme for improvement of quantitative traits. Knowledge of nature of gene effects for productivity and characters related to it, always help in formulating an effective and efficient breeding strategy. In self-pollinated crops, the improvement in yield and other desirable traits is sought by exploitation of additive genetic variance while in case of cross-pollinated crops both additive as well as non-additive genetic variances are exploited. When sufficient amount of genetic variance is present, any selection procedure can be effectively used. For the exploitation of non-additive component which is not fixable, the breeding methodology used are the production of hybrids and synthetics depending upon biological and economic feasibility.

More sophisticated and accurate statistical and mathematical techniques are required for the study of quantitative traits. After Fisher many genetic models based on certain assumptions, were proposed for estimation of gene effects [2]. The majority of these models were created in order to calculate the relative weights assigned to dominance and additive gene effects [3, 4]. It was believed that epistatic gene effects would be minimal. Later studies revealed that epistatic gene effects were present in quantitative traits to a sufficient degree to refute the notion that they were insignificant [5,6,7]. Additional evidence of the presence and the components of epistatic gene effects in the inheritance of various quantitative attributes and their importance in genetic variation is required. Such information would be very useful to a plant breeder in deciding the most appropriate breeding methodology for faster improvements. Generation Mean Analysishas proved to be one of the important techniques to estimate different genetic parameters [7]. The approach based on generation mean analysis, besides being relatively simple in calculation and statistically reliable, has several advantages.

Singh et al. demonstrated the role of both fixable and non-fixable gene effects in characters control in this regard [8]. Additionally, they discovered that the complementary epistasis was not as significant as the duplicate epistasis. Habiba et al. claim that non-additive gene action influenced most of the features for different crossings under the two sites [9]. Non-allelic interactions between additive × dominance and dominance × dominance ruled in the inheritance of seed oil content. Days to maturity, primary and secondary branches/plant, seeds/siliqua, and the oil content were found to be significantly impacted by additive gene effects in six parameter model by Manjunath et al [10]. The results of the study showed that the main raceme length, siliquae on main raceme, 1000-seed weight and seed yield/plant were significantly impacted by both additive and non-additive genes. Based on additive ×additive epistasis for siliqua on main raceme, primary branches/plant, plant height, seeds/siliqua, 1000-seed weight, and seeds yield/plant, Devi et al. reported non-significant variation in Indian mustard; however, dominance and additive genetic components dominated in controlling these traits [11]. While the branches/plant, siliquae/plant, and the concentration of glucosinolate were all significantly influenced by additive effects, the 1000-seed weight was primarily regulated by epistatic effects, as demonstrated by the findings of Bocianowski et al. [12]. In order to speed up the process of genetically enhancing seed yield and other economically significant traits, it is useful to have precise knowledge of the nature and magnitude of gene action for quantitative traits. Other than additive and non-additive effects, epistasis plays a significant role in the inheritance of quantitative traits.In order to determine the nature of gene action that governs yield and its component traits, the current study was conducted. In the current study, two distinct families of B. juncea were used to estimate the effects of various gene action types.

MATERIAL AND METHODS

Six generations of material were used in the experiment: P1, P2, F1, F2, BC1, and BC2. These generations were derived from two PWR 15-8×RGN73 and PWR 15-8×PM25 crosses between the three parental lines, PM-25, RGN-73, and PWR 15-8. Of these PWR 15-8 is a new line developed at Pantnagar through pure line selection from a local germplasm collected from Uttarakhand hills [13]. Two crosses namely PWR 15-8×RGN73 (Family A) and PWR 15-8×PM25 (Family B) were made using selected parental lines. In unreplicated plots, the parents and F1 crosses seeds were raised. These F1 plants were backcrossed with their respective parents (P1 and P2) to yield BC1 and BC2 seeds, and they were selfed to yield F2 seeds. In order to have enough F1 seeds for the final experiment, new F1 crosses were also made at the same time.

The six generations of the two Families were then planted in Compact Family Block Design with three replications. Families were randomly allocated to different blocks within each replication. Different generations of families were randomized within respective blocks. Variable numbers of rows were used for different generations. Non-segregating generations (P_1, P_2 and F_1) were sown in 2 rows each, F_2's in 6 rows, and back cross generations (BC_1 and BC_2) in 4 rows each. The rows were 3.0 m long and 30 cm apart and 15 cm distance between Plants.The following characteristics were measured: number of primary branches and secondary branches/plant, length of the siliqua (cm), seeds/siliqua, days to

maturity, plant height (cm), main raceme length (cm), siliquae on main raceme, siliqua density,1000-seed weight (g) , seed yield/plant (g), oil content (%), glucosinolate content (µmole/g) and protein content (%) were among the traits measured.

In order to assess the effects of genes, the data were put through the joint scaling test and analysis of variance for Compact Family Block Design [14]. The models proposed by Jinks and Jones [16] and Mather and Jinks [15] were used to assess the gene effects. The t-test was used to determine the scales' significance and the gene effects [17]. Agreeance between the results of analyses based on both individual and joint scaling tests was used to determine the suitability of the most suitable model. Two criteria need to be taken into account in order to choose the best or most appropriate model. The estimate of genetic parameters comes in second and the $\chi 2$ value first. The model's adequacy was assessed based on the significance of different genetic parameters and the non-significance of the $\chi 2$ value.

RESULT AND DISCUSSION

Analysis of Variance

Table 1 presents the results of the analysis of variance for all 15 traits that were investigated in two families of Brassica juncea. Day-to-first-flowering, plant height, glucosinolate content, oil content, and protein content were among the quantitative traits for which the analysis of variance revealed that the mean squares resulting from variances within and between families were highly significant. These findings demonstrated that progenies in every family differed significantly from one another. Families with two crosses, containing six generations, also revealed a considerable degree of variation for every character except days to maturity, main raceme length, siliquae on main raceme, density of siliqua, primary and secondary branches/plant, 1000-seed weight, seed yield/plant, siliqua length, and seeds/siliqua.

Gene Effects Evaluation

The type of gene action—additive, dominance, and epistasis—as well as their relative magnitudes determine the breeding strategy to be used for the genetic improvement of the characters. Data were put through joint scaling tests for the estimation of gene effects in order to assess the suitability of various genetic models and for the estimation of different genetic components [18].

The estimates of genetic parameters with standard error under the adequate genetic model for each family with respective χ^2value and the type of epistasis involved in inheritance of all traits are given in **Table 2.**Examination of goodness-of-fit test for different traits showed that an epistatic model was adequate or most appropriate for all the traits in both the families, as simple additive-dominance model was found inadequate in all the cases. These results, thus, revealed that epistasis was an integral part of genetic control of all the traits in the crosses studied. Importance of epistasis for different traits in mustard has been reported earlier by Sachan and Singh, Jain *et al*., Chauhan *et al*., Khan , Kumar *et al*., Kabdal and Singh and Manjunath*et al*. [19,20,21,22,23,24,10].

Table 1: Analysis of variance (mean squares) for Compact Family Block Design for different traits.

S.V.	d.f.	Mean squares														
		DF	DM	PH	LMR	SMR	SD	PB	SB	SL	S/S	TW	Y/P	OC	PC	GC
Replication	2	4.083	27.583	114.427	22.072	20.413	0.002	0.076	0.949	0.015	0.229	0.049	0.546	0.3	0.458	0.672
Family	1	18.778**	5.444	146.576**	5.049	6.922	0.002	0.014	93.929	0.032	16.524	0.174	0.136	2.008**	1.544**	398.038**
Error (a)	2	1.361	2.194	26.67	9.934	4.328	0.007	0.022	2.543	0.009	0.112	0.086	0.106	0.043	0.038	0.523
Progeny	10	34.755**	63.255**	508.31**	286.661**	131.138**	0.173**	0.481**	44.735**	0.353**	2.92**	0.631**	1.951**	0.567**	5.912**	207.997**
Error (b)	20	5.289	12.023	24.166	7.508	7.164	0.002	0.085	1.387	0.074	0.285	0.067	0.253	0.08	0.39	1.346

***significant at 5% probability level** ****significant at 1% probability level**

*Note DF= Days to initial flowering, DM= Days to maturity, PH= Plant Height, LMR=Length of main raceme, SMR=Siliqua on main raceme, SD=Siliqua density , PB=primary branches/plant , SB= secondary branches/plant, SL= Siliqua length, S/S= Number of seeds/siliqua, TW= 1000-seed weight, Y/P= Seed yield/plant, OC=Oil content, PC= Protein Content, GC= Glucosinolate Content.

Table 2.Gene effects for different traits in Indian mustard

Traits	Families	Model	Estimate of gene effects						r^2value	Type of epistasis
			[m]	[d]	[h]	[i]	[j]	[l]		
DF	Family A	4 PM	59.37** ±0.92	4.79* ±1.14	-5.01*± 1.62		-11.50* ±3.46		3.90	-----
	Family B	5 PM	46.62** ±3.75	3.08* ±0.89	15.15± 10.12	9.98* ±3.70		-4.76 ±7.04	0.81	Duplicate epistasis
DM	Family A	6PM	133.17** ±4.857	3.83* ±0.93	-36.50*± 12.31	-9.33 ±4.76	-10.33* ±3.70	21.67* ±8.08	-----	Duplicate epistasis
	Family B	4 PM	113.75** ±1.46	4.95** ±0.64	7.78**± 1.73	10.28** ±2.16			3.55	-------
PH	Family A	5 PM	133.40** ±1.89	14.47** ±1.93		15.20** ±2.87	17.44* ±6.88	32.06** ±2.61	0.98	--------
	Family B	5 PM	152.76** ±1.47	9.17** ±1.47	-62.72** ±7.22		-19.92* ±7.15	62.89** ±6.71	0.21	Duplicate epistasis
LMR	Family A	4 PM	50.56** ±0.72	5.52** ±0.84	-----	-----	-12.25* ±4.05	9.90* ±1.93	4.42	-----
	Family B	4 PM	43.84** ±0.81	13.82** ±0.85	24.97** ±1.74	-----	-28.84 ±5.38	-----	5.21	-----
SMR	Family A	5 PM	40.21** ±1.97	9.52** ±1.03	----	-4.06 ±2.37	-6.60 ±4.88	-0.78 ±3.02	3.61	----
	Family B	4 PM	33.48** ±0.66	4.89** ±0.73	----		-15.62* ±4.22	14.73** ±1.73	1.93	----
SD	Family A	6 PM	0.97** ±0.83	0.27** ±0.01	-0.55± 0.23	-0.21 ±0.08	-0.33* ±0.07	0.25 ±0.15	1.60	-------
	Family B	5 PM	0.91** ±0.03	0.40** ±0.03	-0.91** ±0.11	----	-0.94** ±0.94	0.76** ±0.12	2.70	Duplicate epistasis
PB	Family A	6 PM	3.82** ±0.65	0.28* ±0.09	2.70± 1.54	1.46 ±0.64	-0.19 ±0.40	-2.08 ±0.95	-----	-------
	Family B	4PM	7.39** ±0.72	-----	-7.05* ±1.98	-2.08* ±0.70	-----	4.47* ±1.32	4.35	Duplicate epistasis
SB	Family A	5 PM	23.09** ±1.65	4.95 ±0.66	-9.25** ±2.01	-6.70** ±1.91	4.94* ±2.08		1.57	-------
	Family B	5PM	10.90** ±0.37	1.60* ±0.37	7.45**± 1.68		17.73** ±1.74	-3.82* ±1.62	2.72	Duplicate epistasis
SL	Family A	3PM	3.83** ±0.08	0.51** ±0.08		0.33* ±0.12			0.97	-------
	Family B	5 PM	4.34** ±0.28	0.36* ±0.10	-0.41± 0.40	-0.39 ±0.30	-0.89 ±0.53		3.01	-------
S/S	Family A	3 PM	14.04** ±0.10	1.41** ±0.21			-4.51** ±0.61		7.65	-------
	Family B	5 PM	17.59** ±1.62	-------	-10.21* ±3.81	-5.24* ±1.61	5.65** ±0.87	5.12 ±2.31	0.60	-------
TW	Family A	5 PM	1.99** ±0.05	0.73**±0.11	-------	0.89** ±0.12	-2.08** ±0.36	0.88* ±0.16	0.15	--------
	Family B	3 PM	2.41** ±0.05	0.38* ±0.10	-------		-1.60* ±0.53		3.23	--------
Y/P	Family A	4 PM	5.76** ±0.16	0.83** ±0.17	1.25** ±0.36		-1.64* ±0.58		2.63	-------
	Family B	3 PM	5.75** ±0.14	0.64** ±0.18				1.88** ±0.22	4.55	-------
PC	Family A	5 PM	17.51** ±0.65	1.12** ±0.22	7.93** ±1.00	6.88** ±0.69	2.24 ±0.96	------	2.95	-----
	Family B	5 PM	16.10** ±1.74	0.57* ±0.17	16.30* ±4.77	8.56** ±1.73	------	-8.13* ±3.14	1.38	Duplicate epistasis
OC	Family A	5 PM	38.15** ±0.95	------	5.77* ±2.23	2.03 ±0.94	1.77* ±0.49	-4.03* ±1.37	2.79	Duplicate epistasis
	Family B	5 PM	40.90** ±0.32	0.45* ±0.10	-1.74** ±0.38	-1.09* ±0.34	0.22 ±0.47	------	0.63	------
GC	Family A	5 PM	79.34** ±2.15		32.75** ±5.74	26.40** ±2.08	18.53** ±1.55	-18.23** ±4.10	1.60	Duplicate epistasis
	Family B	6 PM	41.00** ±2.06	5.80** ±0.58	124.88** ±5.33	57.21** ±1.97	10.40** ±1.82	-85.35** ±3.34	-------	Duplicate epistasis

***significant at 5% probability level** ****significant at 1% probability level**

*Note DF= Days to initial flowering, DM= Days to maturity, PH= Plant Height, LMR=Length of main raceme, SMR=Siliqua on main raceme, SD=Siliqua density, PB=primary branches/plant , SB= secondary branches/plant, SL= Siliqua length, S/S= Number of seeds/siliqua, TW= 1000-seed weight, Y/P= Seed yield/plant, OC=Oil content, PC= Protein Content, GC= Glucosinolate Content.

The 6-parameter model was found to be the most suitable, as other digenic interaction models (3-, 4-, or 5-parameter models) proved insufficient for siliqua density, days to maturity and primary branches/plant in Family-A, and for glucosinolate in Family-B. As such, it is not possible to completely rule out the possibility of trigenic or even higher order interactions being involved. It is not feasible to apply a trigenic or more complex model due to the limitations of the current generation. This suggests that genetic control is more intricate for four characters in Family A and one trait in Family B. Higher order interactions for days to flowering and maturity were also reported by Sachan and Singh [19]. The six generations differed from one another, according to the highly significant values of "m" from the generation mean analysis for each of the 15 traits in both Families.

Time to maturity in days

With significant estimates of dominance [h] (-36.50*), dominance × dominance [l] (21.67*), additive x dominance [j] (-10.33*), and additive [d] (3.83*) gene effects, the 6-parameter model was found to be the most appropriate in Family-A. The predominance of duplicate epistasis was indicated by the opposite sign of [h] and [l]. With significant estimates of additive × dominance [i] (10.28**), dominance [h] (7.78**), and additive [d] (4.95**) gene effects, the digenic interaction (4-parameter) model was found adequate in Family-B. The significance of additive and non-additive gene effects for the inheritance of days to maturity is demonstrated by these findings. The results reported by Kumar et al., Kemparaju et al., Mahak et al., Singh et al., and Manjunath et al. are similar to the current findings [23,25,26,8,10].

Height of plant

Regarding plant height, the 5-parameter model for Family-A was found to be sufficient with a significant estimate of the gene effects of dominance × dominance [l] (32.06**), additive × dominance [j] (17.44*), additive × additive [i] (15.20**), and additive [d] (14.47**). Meanwhile, in Family-B, the 5-parameter model was found to be most suitable with a non-significant χ^2 value (0.21) and significant values such as dominance × dominance [l] (62.89**), additive [d] (9.17**), dominance [h] (-62.72**), and additive × dominance [j] (-19.92*). Duplicate epistasis was indicated by the opposing signs of [h] and [l]. Khan, Kumar et al., Ramesh et al., and Kemparaju et al. came to similar conclusions [22,23,27,25].

Main raceme length

The 4-parameter model was found to be adequate for estimating the gene effects for the length of the main raceme in Family-A, as indicated by its non-significant χ^2 value (4.42). The significant estimates of the gene effects for dominance × dominance [l] (9.90*), additive × dominance [j] (-12.25**), and additive [d] (5.52**) were also found. In contrast, the digenic interaction (4-parameter) model in Family-B with significant dominance [h] (24.97**) and additive [d] (13.82**) gene effects found the estimate of χ^2 value (5.21) to be non-significant. These findings highlight the significance of additive and non-additive gene effects for trait inheritance in Family A and additive and dominance gene effects in family B. Yadava et al., Chauhan et al., Singh et al., and Manjunath et al. also obtained similar results [28,21,8,10].

Siliquae on main raceme

Examining the 5-parameter model for the aforementioned trait revealed non-significant χ^2 (3.61) and significant additive [d] (9.52**) gene effects in Family A which indicated the significance of additive gene effects for inheritance of this trait. In contrast, the 4-parameter model detected non-significant χ^2 (1.93) and dominance × dominance [l] (14.73**), additive [d] (4.89**), and additive × dominance [j] (-15.62*) gene effects in Family B. Our results are also in agreement with those of Sridhar and Raut, Kabdal, Mahak et al., and Singh et al. [29,30,26,8].

P**rimary branches/plant**

The estimates of main effects and first order interaction gene effects obtained by applying the 6-parameter model in Family-A demonstrated significant estimates for the additive [d] (0.28*) gene effect in the case of primary branches/plant.In contrast, the 4-parameter model for Family-B revealed substantial estimates for the gene effects of additive × additive [i] (-2.08*), dominance × dominance [l] (4.47*), and dominance [h] (-7.05*). The contrasting [-h] and [+l] indications highlighted the existence of duplicate epistasis. The results of Sridhar and Raut, Kabdal, Gupta et al., and Arifulla et al. are consistent with the current findings [29,30,31,32].

Seeds/siliqua

In Family-A, the digenic 3-parameter model was found adequate. The χ^2 value was non-significant (7.65) and the model detected a significant estimate of dominance [h] (-1.41**) and additive × dominance [j] (-4.51**) gene effects. Whereas for Family-B, the 5-parameter model confirmed its adequacy with a non-significant χ^2 value (0.60).The gene effects of additive × dominance [j] (5.65**), additive × additive [i] (-5.24*), and dominance [h] (- 10.21*) were shown to have significant estimates. These findings demonstrated the role of both fixable and non-fixable gene influences in determining character. The

findings shown by Kemparaju *et al*., Mahak *et al*., and Singh *et al*. are consistent with these findings [25,26,8].

Seed yield/plant

The 4-parameter model's suitability for trait seed yield/plant in Family-A was indicated by the significant estimate of genetic parameters and the non-significant χ^2 value (2.63). The gene effect for additive × dominance [j] (-1.64**), additive h [h] (1.25**), and additive d [0.83**) was found to have significant estimates. The digenic 3-parameter model was found to be sufficient in the case of Family-B. The model identified a significant estimate of dominance × dominance [l] (1.88**) and additive [d] (0.64**) gene effects, although the χ^2 value was non-significant (4.55). These results are consistent with what Kumar et al., Ramesh et al., and Kemparaju *et al.* [23,27,25] have reported.

Oil content

For oil content, adequacy of 5-parameter model was evident due to non-significant χ^2 value (2.79) and significant estimates of genetic parameters in Family-A. For this character, it was determined that the estimates of the dominance [h] gene effect (5.77*), dominance × dominance [l] (-4.03*), and additive × dominance [j] gene effect (1.77*) were significant. Duplicate epistasis was evident when [+h] and [-l] had opposite signals. The non-significant χ^2 value in Family-B, as determined by the 5-parameter model, was 0.63. Significant estimates of the dominance [h] (-1.74**), additive x additive [i] (-1.09), and additive [d] (0.45*) gene effects were found. The present findings are consistent with those reported by [23,25,26,8,10].

The overall results showed that the additive gene effect was significant for days to flowering, plant height, days to maturity, secondary branches/plant, length of siliqua, 1000-seed weight, main raceme length, and protein content in both families; additionally, it was significant for primary branches/plant and seed/siliqua in Family-A only. In both families, the dominant effect was also observed in the following parameters: siliqua density, days to maturity, days to flowering, protein content, oil content, glucosinolate, primary branches and secondary branches/plant. For a wide range of attributes, both additive and dominant effects were therefore significant. However, the relative magnitude of the dominance [h] impact was larger than the additive [d] effect. The observation that epistasis was present in each of these scenarios was fascinating. It has been demonstrated that among the epistatic effects in terms of days to maturity, primary branches/plant, siliqua length, protein content, oil content, and glucosinolate in both families, fixable epistasis—also referred to as additive × additive—is significant. In Family-A, the only significant variables were the siliquae on the main raceme, plant height, siliqua density, secondary branches/plant, and 1000-seed weight; in Family-B, the only significant variables were the days to flowering and seed/siliqua.

CONCLUSION

These results indicate that there exists possibility for improvement through direct selection. As opined by Dickerson selection will have to be deferred to the later generations so to allow the desirable epistatic combinations to establish [33]. Some progress through selection may be expected in early generations as well due to the presence of additive effects. In other situations where non-fixable epistasis in large quanta exploitation of heterosis could be a most appropriate approach.

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CONFLICT OF INTEREST

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